Research

A rapid method to map mutations in *Drosophila*

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Abstract

Background: Genetic screens in *Drosophila* have provided a wealth of information about a variety of cellular and developmental processes. It is now possible to screen for mutant phenotypes in virtually any cell at any stage of development by performing clonal screens using the flp/FRT system. The rate-limiting step in the analysis of these mutants is often the identification of the mutated gene, however, because traditional mapping strategies rely mainly on genetic and cytological markers that are not easily linked to the molecular map.

Results: Here we describe the development of a single-nucleotide polymorphism (SNP) map for chromosome arm 3R. The map contains 73 polymorphisms between the standard FRT chromosome, and a mapping chromosome that carries several visible markers (rucuca), at an average density of one SNP per 370 kilobases (kb). Using this collection, we show that mutants can be mapped to a 400 kb interval in a single meiotic mapping cross, with only a few hundred SNP detection reactions. Discovery of further SNPs in the region of interest allows the mutation to be mapped with the same recombinants to a region of about 50 kb.

Conclusion: The combined use of standard visible markers and molecular polymorphisms in a single mapping strategy greatly reduces both the time and cost of mapping mutations, because it requires at least four times fewer SNP detection reactions than a standard approach. The use of this map, or others developed along the same lines, will greatly facilitate the identification of the molecular lesions in mutants from clonal screens.

Background

Its short generation time and large number of progeny have made *Drosophila melanogaster* one of the best model systems for performing large-scale genetic screens for mutations that affect a given process. For example, the classic screens for mutations that affect the patterning of the embryo led to the discovery of almost all of the genes that control segmentation [1]. Similar screens have identified many of the factors that mediate the development of the nervous system [2], and enhancer and suppressor screens have proved a valuable approach for finding novel components of signal transduction pathways [3,4]. The range of forward genetic screens has been significantly advanced by the adaptation of the yeast flp/FRT recombination system to generate mitotic clones of cells that are homozygous for a particular mutagenized chromosome arm [5]. This technique makes *Drosophila* the only multicellular organism in which it is possible to perform phenotypic screens for mutations that affect the behavior of almost any cell at any stage of development [6-8].

The most common mutagens used in *Drosophila* are P elements, which generate mutations by inserting into genes, and chemicals, such as ethyl methyl sulpfnate (EMS), which modifies bases in the DNA to cause mainly single base substitutions, also known as point mutations [9]. The main advantage of P elements is that it is very straightforward to identify the gene that has been mutated, but P elements are relatively inefficient mutagens. The majority of *Drosophila* genes are predicted to be 'cold spots' for P-element insertion [10], and P-element insertions have been recovered in only a fifth of the genes within a 2.9 megabase (Mb) region that has been thoroughly characterized [11]. This makes P elements a poor mutagen for saturation screens that set out to identify most of the genes required for a particular process. In contrast, chemical mutagens cause a much higher mutation rate, and have less bias in their ability to cause mutations in different loci. The main drawback of these mutagens, however, is that it is not trivial to map point mutations to specific genes, and this is often the rate-limiting step in the analysis.

Two complementary approaches have been standard in mapping point mutations: mapping by meiotic recombination between visible genetic markers, and deletion mapping, in which the mutation is positioned by its failure to complement deficiencies. However, mapping a mutation between widely spaced markers can only give a statistical estimate of its position, and existing collections of deficiencies do not cover the whole genome. Furthermore, the exact positions of many of the visible markers and the breakpoints of the deletions have often only been inferred from cytological and genetic data, and this can make it difficult to link the genetic and molecular maps [12]. Various other approaches have been used to refine the location of the gene, such as P-mediated male recombination [13], or meiotic recombination between two P elements that flank the region containing the mutation. However, several rounds of P-mediated recombination are often necessary to narrow down the region sufficiently for candidate genes to be tested.

In other organisms, which lack the many visible genetic markers and deletions of *Drosophila*, mutations have been commonly mapped using molecular polymorphisms, such as restriction fragment length polymorphisms (RFLPs) or SNPs [14,15]. These have the advantage that they directly link the genetic and physical maps of the chromosome, and can provide a very high density of markers that allow the precise mapping of mutations. For example, the sequencing of the human genome has revealed a very large number of SNPs that cover the genome at a density of one per 1.9 kb, and this will greatly facilitate the mapping of both single-gene and polygenic disease loci [16]. This approach has also been employed to a limited extent in *Drosophila*, but up till now the difficulty and expense of discovering polymorphisms at the DNA level has confined its use to the mapping of mutations within small regions between closely linked markers [17,18].

The recent completion of the *Drosophila* genome sequence [19] makes it possible to search for molecular polymorphisms much more efficiently. It should now be possible to discover enough SNPs to allow the rapid mapping of point mutations within entire chromosome arms. Teeter *et al.* [20] and Hoskins *et al.* [21] have already generated a collection of SNPs that are polymorphic between different inbred wild-type strains. Here we report a high-density map for chromosome arm 3R that has been specifically designed for the rapid mapping of mutations from clonal screens that use the standard FRT chromosome. This approach has two advantages over the use of SNP maps derived from wild-type lines. First, most genetic screens are performed in more complex genetic backgrounds that cannot be traced back to a specific wild-type isolate, and it is therefore more convenient to have a collection of SNPs that can be used directly on the mutagenized chromosomes. Second, by screening for polymorphisms between this chromosome and a standard third chromosome that carries four visible mutations on 3R, we have developed a hybrid mapping strategy that exploits both traditional and molecular markers. This reduces the cost of SNP mapping by a factor of four, and makes it affordable for even small *Drosophila* laboratories. Using this strategy, a mutant can be mapped within two months to a region of about 50 kb with a single meiotic recombination cross.

Results

Method and chromosomes

A number of methods have commonly been used to identify SNPs: detection of single-strand conformation polymorphism (SSCP) by electrophoresis [22], heteroduplex analysis using high-performance liquid chromatography (HPLC) [23], direct DNA sequencing, and variant detector arrays based on differential hybridization techniques [24]. These methods are currently being developed to allow high throughput, cost-effective discovery of SNPs for genome-wide genotyping and population genetics studies. Our aim, however, was to establish a mapping strategy for *Drosophila* that could easily be set up in any laboratory without requiring expensive equipment. We therefore chose the SSCP method because it is straightforward, and allows the rapid detection of a relatively low number of SNPs in a large number of samples.

An important consideration in constructing a SNP map is the choice of chromosomes between which to search for polymorphisms. As clonal screens that employ the flp/FRT technique are becoming more and more common, we decided to develop a SNP map for one of the FRT chromosomes (FRT 82B), as this will be of great use for mapping the large number of mutants generated in these screens. The other chromosome should be as genetically distant as possible from the FRT chromosome, in order to maximize the frequency of polymorphisms. For this reason, the best choice would be a chromosome from a distantly related wild-type

isolate, but these chromosomes carry no visible markers. This means that one has to analyze every recombinant from the meiotic mapping cross with a subset of the SNPs, in order to identify chromosomes in which a recombination event has occurred within the region of interest. We therefore chose instead the widely used *ru h th st cu sr e ca* (also known as rucuca) chromosome which carries four visible markers on 3R. Although this may reduce the frequency of polymorphisms between the two chromosomes, it allows one to select recombinants in the right interval with the visible markers, and dramatically reduces the number of SNP detection reactions required to map a mutation (see Discussion).

Establishment of a SNP map

Pairs of primers were designed to amplify intergenic fragments of 200-300 base-pairs (bp). These primer pairs were scattered through the whole of the right arm of chromosome 3, from the position of the FRT at 82B proximally to the distal tip of the chromosome. The pairs were also designed to have similar melting temperatures, so that multiple fragments could be amplified by PCR in the same block. For each primer pair, PCR reactions were performed on both the marker and the FRT chromosomes, and the PCR products were denatured and run in parallel on an SSCP non-denaturing polyacrylamide gel. When SSCP indicated the presence of a polymorphism, both PCR products were sequenced to determine the molecular nature of both alleles. All the SSCP-positive fragments sequenced (apart from one false positive) contained a polymorphism. 75% of these were single-nucleotide substitutions (SNPs in the strict sense), whereas 25% showed more complicated sequence differences, such as deletions/insertions or changes in several nucleotides (Table 1). The sequences were then analyzed *in silico* to determine whether the polymorphisms created or removed restriction enzyme sites, and more than half correspond to RFLPs. These were confirmed experimentally by digesting the fragments with the appropriate enzyme and running the products on non-denaturing polyacrylamide gels.

As many of the mutations isolated in flp/FRT screens are lethal, they have to be maintained over a balancer chromosome. We therefore tested all newly discovered SNPs on DNA prepared from FRT 82B/TM3, *Sb* and *ru h th st cu sr e ca*/ TM3, *Sb* flies, to ensure that the presence of the TM3 balancer did not prevent the detection of the polymorphism by either method (SSCP or RFLP). In the majority of cases, the DNA fragment amplified from the TM3 chromosome behaved like either the FRT fragment or the marker fragment. However, the distribution of the FRT-like and rucuca-like alleles was very random along the balancer chromosome arm, and a few fragments showed novel migration patterns which indicated that the TM3 balancer carried a third allele.

Figure 1 shows the map of 73 SNPs between the FRT and the marker chromosome. The SNPs are regularly distributed along the chromosome arm, with an average distance between two neighboring SNPs of 370 kb. Table 1 gives a complete list of the SNPs, with the sequence of both alleles, their position on the corresponding genomic scaffold fragments, their approximate cytological locations and the appropriate restriction enzyme when the site also creates an RFLP.

Rate of polymorphism

The average number of polymorphisms between the two chromosomes used in this study is 2.31 per kb, which is probably a conservative estimate of the real frequency as the DNA fragments that did not show a polymorphism on SSCP gels were not sequenced. Figure 2 shows a plot of the percentage of 200-300 bp DNA fragments that contain a polymorphism detectable by SSCP in each 1.5 Mb region of the chromosome arm. Although no conclusions can be drawn from a study of only two chromosomes whose histories are unclear, the frequency of polymorphisms is highest in the center of the arm and decreases near the centromere, which is consistent with larger studies that show that there are more polymorphisms where the recombination rate is most frequent [25]. The one exception to this rule is cytological division 94, which is the least polymorphic region of the whole arm. This suggests that this region of the two chromosomes has a more recent common origin than the rest.

Mapping of a mutant

To determine whether the SNP collection described above is a valuable tool for mapping mutants, we mapped three lethal complementation groups from a germline clone screen on the FRT 82B chromosome (S.G.M., V. Leclerc, K. Litière and D.St J., unpublished data). The crossing scheme for the meiotic mapping of one of these complementation groups is outlined in Figure 3. Following a classical meiotic mapping strategy, one allele of the complementation group was crossed to the marker stock to obtain FRT 82B */ *ru h th st cu sr e ca* females (where * represents the mutation) so that recombination could occur between the two chromosomes (Figure 3, cross 1). These females were then mated *en masse* to males carrying a chromosome with the same recessive visible markers as the marker chromosome and an additional dominant marker *Prickle* (*Pr*), which allows this chromosome to be followed in the progeny (Figure 3, cross 2). Male progeny carrying a recombinant chromosome over the *ru h th st cu sr e Pr ca* chromosome were selected from this cross and scored for the visible markers. Ten recombinants in each of the four intervals between these markers on 3R were then individually mated to females carrying a second independent mutant allele of the complementation group, to determine whether the recombinant chromosomes carried the mutation (Figure 3, cross 3). In the meantime, the other male progeny of the cross were kept at 18°C, so that they could be analyzed later.

From the 40 first individual crosses, we were able to map the mutation between *cu* and *sr*. DNA was then prepared from the ten lines in which a cross-over had occurred in this interval (recombinant/TM3 progeny of cross 3), and was genotyped

Table 1

Molecular data of polymorphisms on 3R

Predicted cytological region	Genomic fragment	Base-pair position within genomic fragment	Polymorphism sequence (FRT chromosome/marker chromosome)	RFLP enzyme	TM3 pattern
82D	AE003605	6135	GCGGTACCTAGAAAC[A/G]GAAATGCTGAAACAG	*Bsl*I	M
		17727	TTCAGATTGCACGCA[G/T]GCGCCACATAGGGTG	*Sph*I	FRT
83D	AE003600	130884	AAAACTATTACAGAA[T/C]TGAGTTTGTGGCTCT	-	M
84A	AE003674	3526	CGGTTAAAATGCCCA[TG/AC]GAGCTCGGAATCCTT	*Sty*I	FRT
		3559	CCCACTTCTTCCCGG[C/T]CCTCATATTTCTTTC		
84B	AE003673	38157	CTGATTTTTATTGTG[C/G]TGCACCCAAGTTTTT	*Apa*LI	M
84D	AE003671	10767	ATAGAGCCATATTTT[A/C]CACATTTTCGTACGG		
		10834	GTTTGTGTTTTTTGT[A/G]CCACGTTATGCATTT	*Rsa*I	M
84E	AE003676	2498	TTCTAGAGGAGGGTG[G/A]GACCTTTCGGAGACG	*Ava*II	FRT
	AE003677	15615	TCTGTGCTGGCAAGC[C/G]TCCCGAGCATTTGCT	-	FRT
84F	AE003679	34211	TGAGCAACGCAAACA[A/C]TTTAATTCAAAAACA	-	Other
85D	AE003683	140539	CTATGAGAATTTGAT[A/T]TCATATAATCGAATT	*Eco*RV	M
85E	AE003684	114149	AAGTAACCATGAAAA[C/T]TGATTCCCCTTGGCT	-	FRT
85F	AE003686	22154	CTGAGTTGAAAATTA[/CCCAAAAAGCCCTGGGCTT] CAATGACAGTCGGCC	*Mva*I	FRT
86C	AE003688	7272	CTTATCTCAAAATTT[/T]GGAAATTTCCCGGGA	-	FRT
		7290	AAATTTCCCGGGAAG[CA/AT]TGCCCGCATGCGCTG	-	
86D	AE003690	3998	CTCTTACATTTTTGG[C/T]CATTTTGGCGGCCCC	*Bgl*I	FRT
		33879	TGTTATTTATAAATA[C/T]GAGGCACATGAATTT	-	FRT
		33960	TCACATACAACTCCG[C/A]CATTTGAGCCCGGAC	-	
86E	AE003692	1660	TGCTGCCTTGAACAC[C/G]TACACGTACAATCGT	*Rsa*I	M
86F	AE003693	19685/19695	TTTTTGCCTTTTGCA[/T] ACGGTCGTA[G/A]TCAGGATGAGAAAAG	-	M
87A	AE003694	44195/44208	GCGAAAGTATTTTTT[T/C]CTTTTTTTTTTT[/T] ATCATTCACAATGGC	-	FRT
		44267	CTAGGGTTGAGTGGG[C/T]GGGTGTAGGATTACC	-	
87C	AE003697	34839	ACATACAAGCGTACA[T/A]AAATTTATTCATATT	-	M
87E	AE003699	18398	TCGATTCAATTCAAT[G/A]ATCAGAACCAACTGT	*Bcl*I	FRT
		18419/18428	AACCAACTGTCTGTG[C/T]TGCTTCCG[C/T] TTCTGGTTGCAACGG		
		18446/18449	CTGGTTGCAACGGGG[C/T]GT[A/C]TGCGTGATGTCTCAC		
		18501	GTGCATAAATTTAAG[C/T]GGGCTGCGAAAAATC		
		24855/24858	TGCAGCCCCCTTATC[C/G]AG[T/C]TGCTGACTGGATTTT	*Taq*I	FRT
		24879	ACTGGATTTTTTTTT[/TTT]GTATTCCGAATCCCC		
		24992/24998	TGGCGGTCTGCGGCG[A/C]AGTCT[C/A]TGCAAACAGGCCGAG		
	AE003700	42096	GCACCTCGTTGCTCG[T/G]CGAATTCTCACGCGT	*Taq*I	M
87F	AE003702	4931/4937	CTGGCACCTACTAAG[A/G]TTCTA[C/T]CGCAAGAACTCCACT	*Hinf*I	FRT
88C	AE003705	2531	TGCTCGGGTTCTCTT[A/G]CCGGCTTTGAAATCG	-	Other
88D	AE003707	14391	TCATCAGGATTCTTT[C/T]GATTTCTTTGCCACA	*Taq*I	M
88F	AE003709	2931/2963	GGCGGTACTAGTACT[GGTACTGGTACTGGTACTGGTACTG/A] GTACTGG[G/T]ACT	ND*	M
89A	AE003711	3499	GCATATAAATTTCCC[G/A]TTCACAACGAACCGC	-	FRT
		3555	GATGGCTTTGTAGTC[T/C]TAAAGTCTCTGAGCA	-	
89B	AE003712	2764	AAGGTGGCTAGGGTG[G/T]TGGATGCAGCTAGAT		
		2809/2820	ACAGGAAGTTCAAAC[C/T]GATAGTGTTA[G/C] CTGTCCATTGATGGA	*Alu*I	FRT
		2836	CTGTCCATTGATGGA[T/G]GATTTTATATACGAT		
		2895	CTTTGCGGTAGGAAA[C/T]CAAGATGAAAATGTC		

Table 1 (continued)

Predicted cytological region	Genomic fragment	Base-pair position within genomic fragment	Polymorphism sequence (FRT chromosome/marker chromosome)	RFLP enzyme	TM3 pattern
89C	AE003713	20964	TTCCCATTCACATTC[A/G]CACATGCTGGGCACG		
		20984	TGCTGGGCACGAGTA[C/T]TCGCCGATATCGATC	*Sca*I	M
89E	AE003715	299	CTAATGGCTCCTACG[A/G]CATTTTGCTTTCTTC		
		320	TGCTTTCTTCGAATG[T/A]TTCTGTTACCAAACT	*Hinf*I	FRT
		356	GCTCCGCTCCCTCCA[T/C]TTGTATCTGTCGGAT		
90A	AE003716	214757	AAAATATGTTGTACT[AG/GTTGTT]GAATGAAAAGTAAAA	-	FRT
		214807	ATTTTATCTATCGGG[C/A]TACTAATGGAATCTG	-	
		214862	CTAATATCTAATGCT[T/A]AGCTACAATATGATG	-	
90C	AE003719	15267	ATCGCCTACTAAATG	ND*	FRT
			[GACCCAATGCCGATCCGTTTCGAGCACTTGCAGTTG/]AT…		
		15306	G[G/A]AGCT[A/G]TTGGCTGTT[GAC/AAT]TGCGA		
			[A/G]GATAACACACAGGAG		
90D	AE003720	2929	AGTTCGATTCAGTCG[C/T]TTTGGATAGAGCTCA	-	FRT
		3053	ATTTCAGATGGGAAA[G/C]GCATTAGATCTTGCT	-	
91B	AE003723	6105	AAGTGTGCTCTGTTT[C/T]TCGTTTAAGTTACGC		
		6202/6208	GTTAAAGCTACAGTT[T/G]CAGTT	ND*	M
			[ACAGTTTCAGTTACAGTGA/T]CAGTTACAGTT		
		6340	AGTTATACCCCACTT[CACTT/]GCTCGTTTTAATAAT		
91D	AE003724	200	GCACACACACACACA[CAGTT/GGT]ACTGCCCCGCCCACT	*Rsa*I	FRT
91F	AE003725	11172	TTATTATGACAACAT[G/C]CATCCATTAGACCCA	*Nsi*I	FRT
92A	AE003726	519	CTCGTCTCCCTGATC[G/A]CAATGGTAGTGGAAA	*Bcl*I	M
		565	TAATAATGGTAATGG[CAATGG/]TAATGGTAATGGTGA		
		608	CCGCCCCAACACAAC[C/T]GAGTTCGTGAGTGCG		
92D	AE003729	7430	CATTCAATTGGTGGG[/G]ACCGCTTTTGCCCAA	-	ND
92E	AE003730	9025	GGCTAATCCGTAATC[/CGTGCCCGTACCCGTACCCCTACC]	ND*	FRT
			CGTACCCCTACCCGT		
93B	AE003733	759	GTCCTTCAAGGCAGA[/TATGGA]TATGGAAGTGCATAA	-	Other
93D	AE003734	25325	AAAGCTAATTGATTT[C/G]CATGAAAGTGAGCCA		
		25391	TGAACTCGCTGCTCT[C/T]CCACTTATATACATC	*Ear*I	FRT
93F	AE003736	2695	AAACTTCAGCAACTT[C/A]AGCCGCCAACAACAA	*Mse*I	FRT
	AE003737	587	TTTGCCGTTGAAGCT[GCAGCAGCAGCAGCA/]	-	Other
			GCAGCAGCAGCAGCA		
		722	ACTTTGCCAACTTCT[T/]GTGCCTTTTTGTGCC	-	
		144616/144630	GATGCCCTACCCCCT[T/C]CTTCACTTTCGCT[AGA/TGC]	-	M
			AAGGGGAATGTAAAA		
94A	AE003738	687	GTGGATGATGATGCC[/C]GGTTGGTGCGTTGTT	*Scr*FI	M
95A	AE003743	66247	TGCAAAAAAAAAAAA[T/]ATATAGCTTGGCTAC	-	M
95B	AE003744	73639	ACCGAACCGCCCAGC[/AC]CACCCACTCAACCG	-	Other
		98594/98607	GGACAACAATTGTAC[T/]CCCCCCAAGTAC[A/C]		
			CCACGAAAATTTCAT		
		98678	AATCTTGAACTCTAA[A/G]CTGGCACGTTAAGTT	*Alu*I	FRT
		98714/98729	AAATAATTGTATTTT[T/G]TGTTTTATCCGTTT[CT/TTC]		
95D	AE003746	239	ACAACTAGTTAAATC[C/A]AATTTCGAGCAGAGA		
			GTGAAGAAACTGGTT		
		313/323/329	TGCATTTTATCGCAT[C/T]CCCCCTCCC[C/T]CCCTT[G/A]	*Mse*I	FRT
			AGGCTAACAACTTTG		
		351	ACAACTTTGGACAAC[A/G]TCCGATCATACGCAT		
96A	AE003748	492	GATGTTGCTGCTGCC[T/G]TTGCTGCTGTTGCCG	-	M
		631	TCCATCAGACAGCAG[/CACACCAC]CACACCACCACCAAA	-	
96B	AE003750	11903	AACCAATCGCAACAA[CTT/GTC]GAATTATTATTTATT	-	Other
		12039	GACGCGTCATCACTC[C/G]GCCGGCCACCTGTGG	-	
		13160	TTTTCGCTGCCCCCC[/C]ATCCCAATTCTTCTG	-	ND

Table 1 (continued)

Predicted cytological region	Genomic fragment	Base-pair position within genomic fragment	Polymorphism sequence (FRT chromosome/marker chromosome)	RFLP enzyme	TM3 pattern
		13198	CGCAGTTCTTCGGAC[A/T]TCTCGCTCCCTACGC	-	
		13259	CGATCTATCGATCGG[G/A]CGATCGAACTGTAAC	-	
96F	AE003753	8873	TTGTCAACGTGGTTG[A/C]ACTCACTTGGCGACC	-	Other
97B	AE003756	204	TAAAAATGGCAGAGG[A/G]CAATCCGCAGTTCCC	-	ND
		300	TTGCCTTCGTCTAAG[A/G]ACACAAGGGGTTCTC	-	
		1673	CTCCGTCCTTCAGTC[/CTTCAGGC]AGTCAGTCAGTCAGT	-	M
97C	AE003757	8170/8177	CTCTTCCTAAAGATG[C/A]TAGTTT[/T] AAGGATAGTTTGCGC	-	M
		8245	TTTATGATTGCGTTG[G/A]GCATAAAAATGTGAA	-	
		8269	ATGTGAAATATTGTT[A/G]AAGCCGAGACATTAA	-	
		8883	TCACGCTCTCCTGGC[C/T]AGGATGCACATTTGC	*Hae*III	FRT
97F	AE003760	104426	TCGGTTTCGGTTTTG[C/T]CCCACTGAAAAACAT		
		104483	CGAAAAATTGGTATT[C/T]AACTAATGGGCAACG	*Mse*I	M
98A	AE003761	1047	CACACGAAACGTAAA[C/G]GATGCGCAGCACTTA	*Fok*I	M
98B	AE003762	1805	CACAATCCCAGCTCG[G/A]GTTATATAAGTCTGT	*Xho*I	FRT
	AE003763	1291	GCTTAACAAGTTGTG[G/T]CAATTGGTCGGCAAA	-	Other
		3570	ATAATAAAATAAAAC[T/C]CAATATCCTGGCGAG		
		3628	GCATGTGAACTATTC[G/A]ATTCGCTCATTTCAT	*Hinf*I	M
98C	AE003764	1318	GATAACTATATGGAT[G/T]GGATATATGGCAGGC	*Fok*I	M
98D	AE003767	38575	GGGGAGGAAAAGCGA[T/C]TTTTCTACTTATTTT	-	M
98E	AE003768	13811	ATTGTCGAAACTGCT[G/A]GCTTCAAATTGAATT	-	ND
		13877	TCAAGCTCCACGCCT[G/A]TTGGAATTCATTTAT	-	
99A	AE003769	3234	AACACATTCCCTGTG[A/G]CCACCCAAGCATCCC	*Hae*III	M
		3333	AAACTTGAACTCGAA[C/A]TGGTACGGCGAAGTG		
99C	AE003771	43332	AACTATCGAGCTGGT[AAAAC/]A(13)		
		43486	ATACGTTTATCAGGA[G/C]CGC…		
		43492	TG[TTTATAGCGAAAATGGGGGAGGGGGGGGTT/ AGAGGGGGGTG]GGGGTTAGAT	ND*	FRT
99E	AE003773	228	GTGAACTTGGCACTG[C/A]GGCGTGACTCAGAAA	-	M
100B	AE003776	497	TTCCTCCCCTAAAAG[A/C]ACCGACAAATGCAGT		
		519	AAATGCAGTAGTTAG[C/G]CCAAGACATCATCTC	*Hae*III	M
	AE003777	451	AATGTTGGCAGGACC[T/A]CTCGATGAGTTGGCT	*Dra*II	FRT
100C	AE003778	1549	AGAAAACCGCAGGGC[G/T]TGTGGCGCATTTCGA	-	FRT
100F	AE003780	60202	TTACAAATTTTTTTT[/TT]ACCTAGCTTACAATT	-	FRT
		85827	ACATAATGAAGAGAC[T/C]ATTTGAAATATTCAT	-	Other

All the polymorphisms analyzed between the FRT 82B chromosome and the marker chromosome are shown. The table lists the approximate cytological location, the accession number of the genomic scaffold sections, the base-pair position within the section and the sequence of the polymorphisms. In 75% of the cases, the polymorphisms are real SNPs, where one nucleotide is replaced by another. In the other 25%, the nature of the polymorphisms is more complex, and involves multiple base changes or small deletions/insertions. When the polymorphism creates an RFLP (in about 50% of the cases), the experimentally tested enzyme is shown on the right. In four cases, a 2% agarose gel could resolve the migration difference of the two PCR products (when the polymorphisms were deletions of at least 20 bp). In these cases, RFLP patterns were not tested (indicated by ND*). The last column indicates the pattern of the TM3 balancer chromosome on either RFLP or SSCP gels. M indicates that the TM3 band co-migrates with the marker chromosome and FRT, that it co-migrates with the FRT chromosome. In a few cases, the TM3 migration pattern was different from both of these chromosomes, indicating the presence of another allele on the balancer.

using SNP-RFLPs from the map described above. To reduce the number of RFLPs that needed to be analyzed in each line, we first determined whether the mutation lay to the left or right of an RFLP in the middle of the *cu-sr* region, and then mapped the five lines in which recombination had occurred in the same interval using a second SNP-RFLP in the middle of the new region. By repeating this process with four further RFLPs, the mutation was mapped to an interval of about 2 Mb between the two closest RFLPs (Figure 4, step 1).

To refine the mapping further, we identified 126 additional males in which recombination had occurred between *cu* and

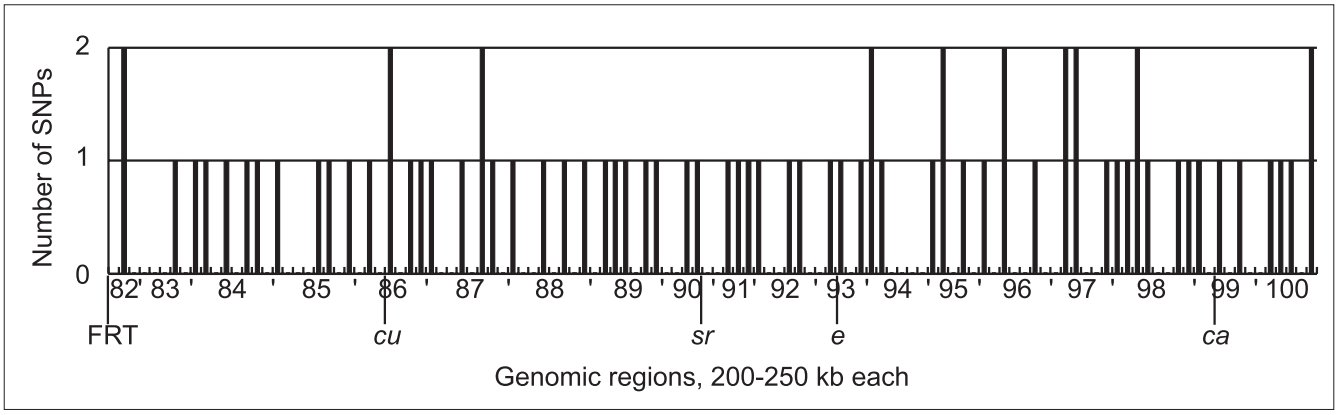

Figure 1
A SNP map for chromosome arm 3R. A plot of the polymorphisms found on 3R is shown. The *x*-axis is approximately co-linear with the molecular map of the chromosome, with each region representing one section of the genomic scaffold. The estimated cytological positions are indicated underneath. The *y*-axis shows the number of polymorphisms found in a given region. For a detailed description of the individual polymorphisms, see Table 1. Seventy-three polymorphisms are distributed along the whole arm, with an average distance between them of 370 kb.

sr from the flies that had been stored at 18°C. These males were again individually crossed to females carrying the second allele to score for the presence of the mutation, and DNA was prepared from the recombinant/TM3 progeny of this cross for genotyping with the two closest RFLPs defined above. This allowed the identification of 45 informative recombinants, in which a cross-over had occurred between these RFLPs, and the other recombinants were discarded (Figure 4, step 2). Genotyping these recombinants with the two remaining SNPs in this interval placed our mutation in a 400 kb region (Figure 4, step 3).

At this point, the complementation group had been positioned between two closest SNPs on the map described in Figure 1, but there were still eight remaining recombinants in this interval. We therefore screened for more SNPs in this region in order to map the gene more precisely, and identified six polymorphisms on SSCP gels from 18 primer pairs tested. These polymorphisms allowed us to map the mutation to a 47 kb interval that contains nine genes, according to the Berkeley *Drosophila* Genome Project (BDGP) annotations [26]. Three of these genes are not required for viability and can therefore be disregarded as our mutation is lethal, while lethal P-insertions are available for two others, making it possible to test them directly by complementation. This narrowed the complementation group down to four predicted genes, one of which appeared to be a good candidate on the basis of the function of its homologs in other species, and sequencing revealed that both alleles contain mutations that disrupt this coding region. Thus, we were able to map the mutation to a single gene in less than two months, using one mapping cross that generated about 1,200 recombinant chromosomes.

Two other mutations were mapped using the same strategy. Using 147 recombinants in the *e-ca* region, a second complementation group was mapped to a 60 kb interval containing 16 predicted genes, whereas the third locus could not be mapped precisely because we recovered no recombinants in a region of over 300 kb. This suggests that the allele chosen for mapping carries a chromosomal rearrangement, such as an inversion, which suppresses recombination in this region, but it should be possible to map this locus more accurately using another independent allele.

Discussion

In this paper, we describe the development of a SNP map of chromosome arm 3R between the widely used *Drosophila* chromosomes FRT 82B and rucuca, and demonstrate that this map can be used to map mutations rapidly and accurately. One essential requirement for efficient mapping is the availability of a high enough density of polymorphic markers. Although these stocks were not chosen for their diversity, the average frequency of SNPs between them is 2.3 per kb, and even the least polymorphic region has more than one SNP every 2 kb. As the *Drosophila* euchromatin contains one gene every 9 kb on average, it should therefore be possible to find more than enough markers in any region of the chromosome to map a mutation to the level of a single gene. The frequency of SNPs that we obtained is slightly less than half that observed by Teeter *et al.* between pairs of wild-type chromosomes [20], but within the range observed by Hoskins *et al.* [21]. Although the full pedigrees of these two chromosomes are unknown, the recessive visible mutations on the rucuca chromosome were all discovered in Thomas Hunt Morgan's laboratory in the first quarter of the twentieth century, whereas the FRT 82B is inserted on a ry^{506} chromosome that was derived from an unspecified wild-type stock in 1972 [27,28]. Thus, these stocks probably originate from different wild-type isolates, and have been

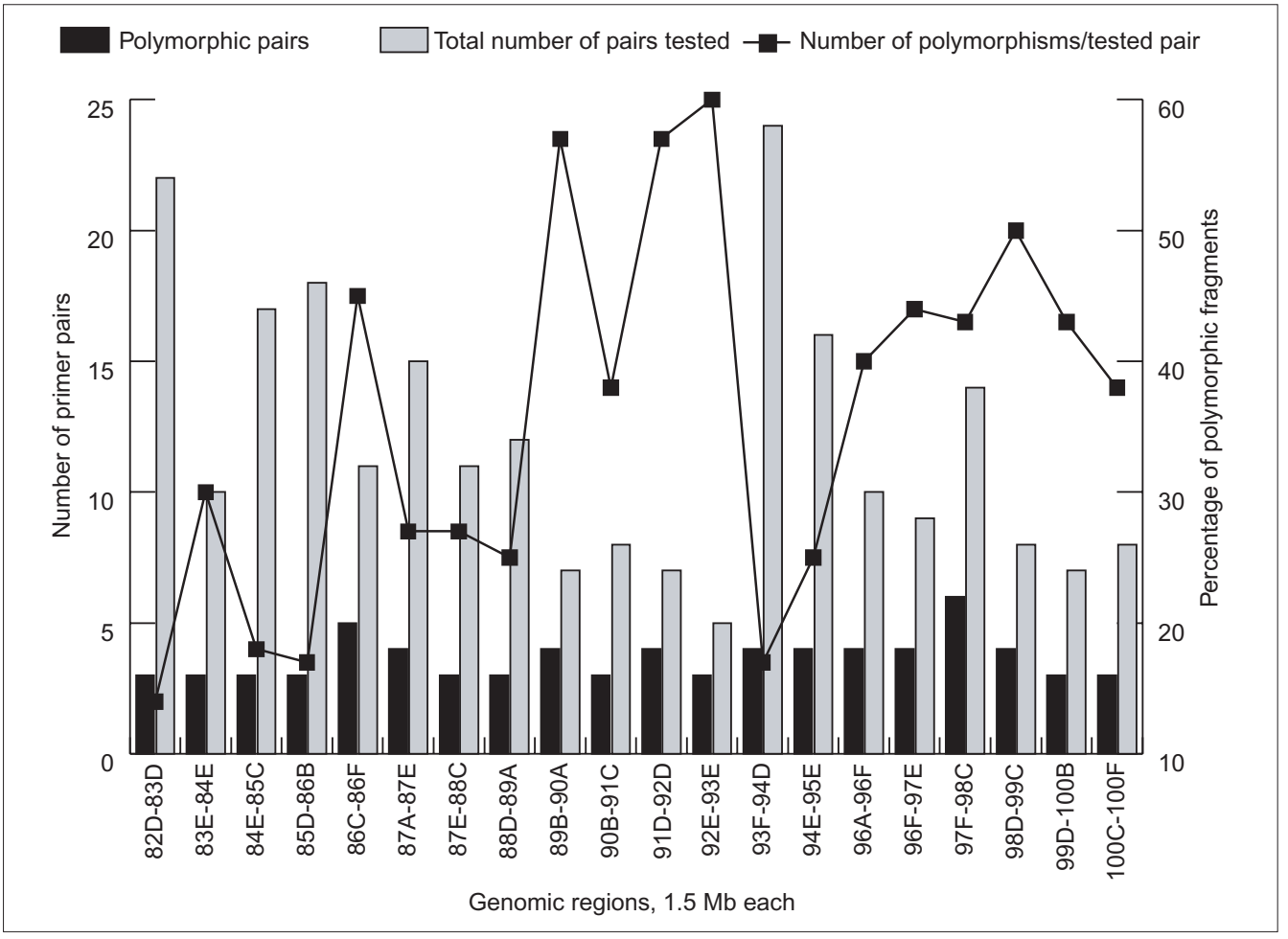

Figure 2
The frequency of polymorphisms varies along the chromosome arm. Black bars indicate the number of 200-300 bp DNA fragments tested that are polymorphic between the FRT 82B and the marker chromosome as detected by SSCP; gray bars show the total number of DNA fragments tested. Black squares show the percentage of DNA fragments tested that are polymorphic out of the total number of fragments tested. The *x*-axis is co-linear with the molecular map of the chromosome, with each region representing about 1.5 Mb (six genomic scaffold sections). For convenience, the correspondence to the cytological map is indicated below. A linear regression of the percentage of polymorphisms (data not shown) confirms that the frequency of polymorphisms decreases towards the centromere. Cytological region 94 shows a strong unexplained dip in the frequency of polymorphisms.

separated in the laboratory for at least 80 years. Preliminary analysis suggests that there is a similar rate of polymorphism between other well-separated pairs of laboratory chromosomes, such as the standard mapping stock for the second chromosome, *al dp b pr c px sp* and the FRT 40A chromosome used in flp/FRT screens on 2L, and between this mapping stock and a *cn* chromosome that we have used in an enhancer screen (J.-R. Huynh, I. Torres and D.St J., personal communication). Thus, it should be possible to discover sufficient SNPs between other pairs of laboratory chromosomes to use this strategy for mapping mutations that have been induced in different genetic backgrounds.

The accuracy of SNP mapping depends on the number of recombinants analyzed. We performed our mapping crosses in 12-15 bottles, and recovered about 1,200 recombinant males of the right genotype in each case; this should allow mapping to a resolution of about 0.1 centimorgans (cM). As the right arm of the third chromosome spans 60 cM, and contains 28 Mb of DNA, 0.1 cM corresponds to an average of 46 kb. Although this will vary along the length of the chromosome with the rate of recombination, our results from the mapping of two mutations in different regions are consistent with this estimate. In each case, we were able to map the mutation in a single mapping cross to a interval of approximately 60 kb. This level of resolution will usually be insufficient to map the mutation to a single gene, and a second round of mapping is often required. One efficient way of doing this is to select for recombinants between closely linked P elements, but one still has to screen at least an

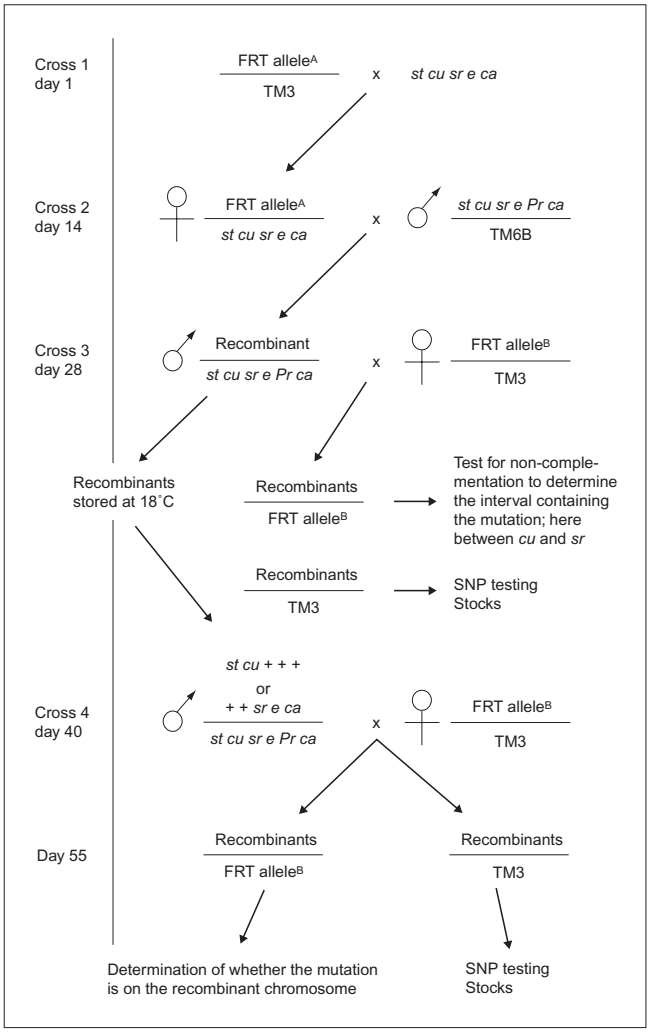

Figure 3
Crossing scheme for mapping a complementation group. The mapping crosses follow the typical design for mapping one allele (A) of a complementation group on the basis of its failure to complement a second allele (B). The first cross generates females that are heterozygous for the FRT chromosome carrying allele A and the marker chromosome, so that meiotic recombination can occur between them (cross 1). These females are crossed to males carrying a dominantly marked (*Pr*) marker chromosome (cross 2) so that all of the recessive markers can be scored in the recombinant progeny to determine in which intervals recombination took place. A small number of recombinant males (10 in each interval) are then individually crossed to females carrying the second allele (B) to determine whether the recombinant chromosomes carry allele A or not (cross 3). This allows the mapping of the gene between two visible markers, in this example, between *cu* and *sr*. The progeny of cross 3, carrying both the recombinant chromosome and TM3 are then tested with SNP-RFLPs to narrow down the region containing the mutation. More males recombinant between *cu* and *sr* are then selected from the rest of the progeny of cross 2, that have been stored at 18°C. These males are then individually crossed to females carrying the allele B (day 40, cross 4). Progeny of this cross are used to determine whether the recombinant chromosomes carry allele A, and to genotype the recombinants with SNPs. The recombinants with a cross-over in the same small interval as the mutation are kept as stocks over TM3, for more precise mapping with further SNPs, once these have been discovered. For convenience, only visible markers used in the mapping are indicated in this figure.

order of magnitude more recombinants to narrow the region down to around the size of the average locus. As a region of this size should contain only a small number of genes (typically fewer than ten), in most cases, it is more efficient to test them individually by other means. For example, if the mutation produces a clear embryonic phenotype, the genes can be screened by RNA interference to determine which double-stranded RNA phenocopies the mutant phenotype [29,30]. An alternative, which we used successfully for our first complementation group, is to sequence candidate genes to search for molecular lesions in mutant alleles.

The mapping method described here has two major advantages. First, it is very quick, as the whole process takes only two months, and a precise map position can be obtained within days of determining which recombinant chromosomes carry the original mutation. More importantly, the method is also inexpensive. In the example outlined in detail above, the initial mapping of the mutation to a 400 kb region required 210 PCR reactions, 207 RFLP digests and 3 SSCP reactions, and cost less than £100 (£0.185 per PCR reaction, £1 per SSCP lane and £0.22 per RFLP detection). Even though the more precise mapping required the discovery of six further SNPs, this only doubles the cost per mutant. Furthermore, the SNP map becomes denser each time a mutant is mapped in this way, and the cost and time of mapping subsequent mutations in the same region is therefore reduced.

Most SNP maps that are being developed in *Drosophila* are between chromosomes from wild-type stocks that have been chosen because of their diversity. Although these chromosomes will be invaluable for mapping quantitative trait loci in wild-type populations, they do not carry any visible markers, and this significantly increases the time and cost of using them for mapping mutations. For example, a mapping experiment with 1,000 chromosomes would require more than 2,300 SNP detection reactions, because every chromosome from the cross needs to be tested for at least two SNPs to determine where recombination occurred. In contrast, only about 300 SNP detections are required to perform the same experiment with the multiply marked chromosome. This also reduces by a factor of four the number of single fly DNA preparations, one of the most tedious and time-consuming parts of the procedure.

In a few cases, the use of visible markers will not help in the mapping of the mutation. If the mutation lies distal to the last visible marker on the chromosome (in this case *ca*), there is no way to select visually for recombination events between this marker and the end of the chromosome. In this

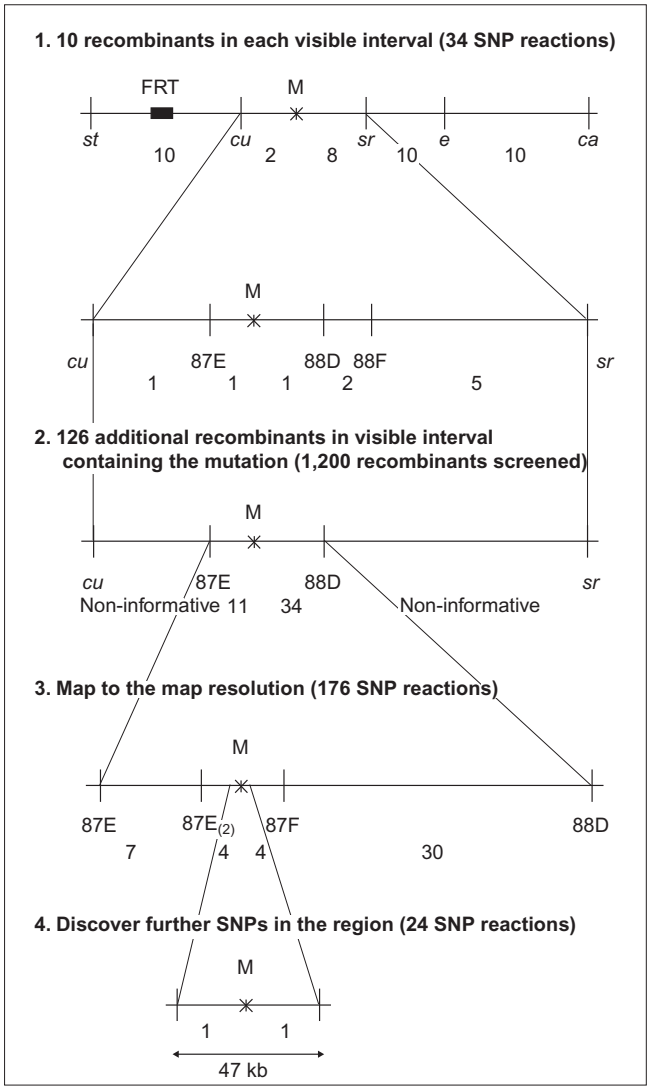

Figure 4
Overview of the mapping of one mutant. Step 1: The mutant was mapped between the visible markers, using ten recombinants in each interval, and SNP-RFLPs were used to refine the map position. Step 2: We collected more recombinant males in the previously determined visible interval and tested them with the RFLPs flanking the mutation, as determined in the first step. This allowed us to find 45 informative recombinants. Step 3: These recombinants were further tested with SNPs from the map. This placed our mutation in a 400 kb interval, with eight recombinants still available. Step 4: The last step involved the discovery of more SNPs in that same interval and the fine mapping of the gene. We were able to map it to a 47 kb region. The numbers below the schematic maps of the chromosome indicate the numbers of recombinants in the given intervals.

case, one has to screen for recombinants between *ca* and the tip of the chromosome, using a very distal SNP, as one would do with a non-marked chromosome. Based on the size of this distal region, this should occur less than 10% of the time.

This mapping method with marked chromosomes can be used in any laboratory without the need for a specialized infrastructure. Using the chromosomes and the map described here, RFLPs can be used immediately and the remaining SNPs can either be detected on an SSCP system or by sequencing. It should also be straightforward to develop a SNP map for other pairs of chromosomes, which can then be used to map multiple mutants from screens that have been performed in different genetic backgrounds. The construction of the map presented here required 300 pairs of primers and SSCP detections, and 150 sequencing reactions, and took about three months at a cost of about £4,000. An alternative strategy, which does not involve the use of an SSCP detection system, is to discover the SNPs by sequencing. With a SNP frequency of 2 per kb, it is very likely that every kilobase of DNA sequenced will reveal at least one polymorphism, and this will reduce the number of primers required. As over half of the SNPs actually create an RFLP, one cost-effective strategy would be to ignore the other SNPs and use only the RFLPs for mapping mutants.

In some cases, such as screens for mutants with behavioral phenotypes, it is too time-consuming and expensive to perform large-scale saturation mutagenesis, and this results in the recovery of many mutations that do not fall into complementation groups. Because the mutant chromosomes recovered from screens are often heavily mutated, there is always a risk that the phenotype of a single mutant chromosome is synthetic, that is, the result of several interacting mutations. Similarly, when the mutant chromosome is lethal, it is very difficult to find out whether the lethality is due to the same mutation that causes the phenotype of interest, or to an unrelated hit on the chromosome. SNP mapping provides a simple way to resolve these issues, because mutations can be rapidly mapped to a region of approximately 60 kb. If a mutation that causes the phenotype can be mapped to such a small region, one can almost completely exclude the possibility that the phenotype is the result of two independent mutations. In the same way, any lethal mutations on the chromosome can be mapped and tested for the original phenotype. If the lethality and the phenotype map to the same region, one can be confident that they are caused by the same mutation. The convenience and speed of this approach therefore makes it particularly suitable for mapping single alleles.

Given how valuable such a tool would be for the whole *Drosophila* community, ideas are being discussed amongst *Drosophila* workers on how to develop freely available SNP databases that contain a much higher density of SNPs between these chromosomes and several others. Access to such databases, along with the availability of primers or a mapping service, would greatly facilitate the mapping and cloning of mutations, and would make *Drosophila* an even

more attractive model system for forward genetic screens for studying many aspects of biology.

Materials and methods

Drosophila stocks

FRT 82B: w^-; P[ry^{506}; hs neo; FRT] 82B [5]. Marker: *ru h th st cu sr e ca*. Dominantly marked chromosome: *ru h th st cu sr e Pr ca* / TM6B. Balancer: TM3, *Sb*, kept as w^-; *Pr Dr* / TM3, *Sb* [31]. All four stocks are available from public stock centers, but the flies used in this study have been kept in the laboratory stock collection for a number of years, and it is unclear how quickly polymorphisms develop in laboratory strains. All stocks were maintained on standard *Drosophila* food.

DNA preparation

Individual flies were mashed up in 50 µl of a 10 mM Tris-Cl pH 8.0, 1 mM EDTA, 25 mM NaCl, 200 µg/ml proteinase K solution, incubated at 37°C for 20 min and denatured at 95°C for 1-2 min to inactivate the proteinase K. DNA preparations were kept at 4°C.

PCR

Primer pairs were designed to have an annealing temperature of about 58°C using the MacVector 7 software (Oxford Molecular plc). A 20 µl reaction containing 1x NH_4 BioTaq-buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.25 U BioTaq, 500 nM primers and 1 µl template DNA was subjected to the following cycle: 30 sec 95°C, 30 sec 58°C, 30 sec 72°C repeated 30 times.

SSCP gels

Two microliters of the PCR reactions were mixed with 2 µl of a 95% deionized formamide, 20 mM EDTA denaturing loading buffer. The samples were denatured for 5 min at 95°C and immediately cooled to 4°C in an ice-water bath to prevent reannealing of the denatured single strands. They were loaded on precast 12.5% or 20% homogeneous non-denaturing Phatsystem polyacrylamide gels (Pharmacia) and were subjected to electrophoresis at 10°C. They were stained with the Pharmacia silver staining kit in the development chamber of the system, following the manufacturer's protocol [32].

RFLP gels

Four microliters of the PCR products were digested with the appropriate enzyme. The digested samples were then loaded onto a non-denaturing polyacrylamide gel (10% 29:1 acrylamide:bisacrylamide mix, 1x TBE, 5% glycerol) and run in 1x TBE buffer.

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