Table 2. Tissue variation of arylsulfatase C

Tissue	SJL/J	A/J
Liver	2.51±0.17	1.02±0.15
Kidney	0.71±0.07	0.17±0.06
Brain	0.25±0.02	0.10±0.01
Testis	1.20±0.10	0.55±0.10
Spleen	0.59±0.04	0.48±0.08
Lung	0.40±0.12	0.31±0.04
Heart	0.14±0.03	0.08±0.01

Activities are expressed as μmoles/g/h; mean of 3 animals ±SEM.

Table 3. Biochemical properties of arylsulfatase C

	Liver	Kidney
pH optimum	8.7	8.2
Temperature optimum	46 °C	43 °C
$t_{1/2}$ 55 °C (min)	37	7
K_m (mM)	0.65±0.15	1.58±0.15
Inhibition (%)		
5 mM *p*-nitrophenyl-SO_4	53.5	
5 mM Na_2SO_3	45.9	
5 mM Na_2SO_4	10.8	
200 mM NaH_2PO_4–Na_2HPO_4	28.0	

Strain survey. Liver aryl C activities for 26 murine strains are presented in table 1. SJL/J and A/J mice were selected as representatives of high- and low-activity strains, respectively. 2-fold or greater differences of aryl C activity were observed in liver, kidney, brain and testis. Variation of spleen, lung and heart aryl C activity appeared more conservative (table 2).

Developmental profiles. Developmental variation of aryl C activity is illustrated in the figure. Liver aryl C activity peaked at approximately 10 days, declined and increased to adult levels at 50 days of age. SJL/J aryl C activities were significantly higher than those of A/J mice at all stages tested after day 1. Developmental profiles for brain and kidney aryl C activities were similar to those observed in liver.

Biochemical characterization. The general biochemical properties of SJL/J and A/J kidney and liver aryl C are summarized in table 3. No significant interstrain variation of these properties was apparent. However, the kidney enzyme possessed a higher K_m and was more thermolabile than the liver enzyme in all strains evaluated. These differences suggest that distinct enzymes occur in these 2 tissues or that post-translational processing of aryl C may differ in liver and kidney.

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Genetic validation of a *Drosophila* learning task

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Summary. 4 inbred strains of *Drosophila melanogaster* and crosses between them were tested in 2 types of multiple-choice T-maze. It is suggested that genetic analysis can distinguish learning from other behaviours implicated in maze performance. Directional dominance for high performance, which is characteristic of learning in many species, was found only for those aspects of behaviour previously hypothesised as involving learning.

The ability of *Drosophila* to learn has been demonstrated with several different paradigms[1-3], opening the way to detailed genetic analyses of learning. However, if *Drosophila* are to be used as a general model of learning and memory, it is first necessary to show parallels between their behaviour and that of higher organisms. It is already known that cooling[4] and cycloheximide[5] disrupt memory in *Drosophila* just as in vertebrates. The present paper describes a genetic similarity, in that there is directional dominance on a *Drosophila* maze task, similar to that found for rodent learning[6]. Secondly, because this dominance is found only for learning and not for alternative behaviours which critics[7,8] have suggested might explain performance in the mazes, this task is further validated as a measure of learning.

This experiment involved four strains of *Drosophila melanogaster*, their F_1s and the intercross between these F_1s. From 10 Leslie Manor (LM) strains, described and tested previously[3], LM 20, 26, 27 and 28 were chosen as covering the complete range of variation from learning to no-learning. For the F_1s, reciprocal crosses were made of LM 20 with 26 and of 27 with 28. Because of the number of flies available, the intercross generation involved only the 2 reciprocal crosses of (26×20) with (28×27). The subjects were 8 samples of 300 flies (150 male and 150 female) from each of the 4 strains and the 4 F_1s and 16 samples from each of the 2 intercrosses, to give an identical number of flies (9600) tested from each generation. All testing was done at 25 °C on flies 3 days after eclosion, with testing beginning at the same time each day to avoid the problem of circadian rhythms of activity[9].

4 of the 8 samples of each type were tested in the 'forced-choice' maze used previously[3] to study learning. This is a 10-choice multiple T-maze of the kind normally used to study taxes, but modified with passages blocked off, so that after initially going left or right (no differential light or gravity cues are provided) the flies are forced to make a sequence of 5 left or 5 right turns, depending on their initial choice. Then they are given a second choice, forced to make another 2 turns and given a final choice. The learning explanation of the performance in this maze is that within 1 run in the maze, the flies associate the sequence of turns they have to make with progress through the maze towards a battery of lights behind the end tubes. The only obvious reinforcement is the opportunity to make their normal positive phototactic response. The criterion of learning is

whether or not the flies, when they reach the second choice point, repeat the turn which they have been forced to make at the 5 previous junctions in the maze. For practical convenience, the flies have 24 h to complete the maze but most reach the second choice point in less than 4 h, well within the retention span of *Drosophila* on at least 2 other learning tasks[2,5].

The remaining 4 samples served as a control, being tested in the conventional maze with no passages blocked off and where all 10 choices were available. The layout of both types of maze is shown in the figure.

Results and discussion. In the absence of any consistent significant differences, the data have been averaged over sexes and over reciprocal crosses (in the case of the hybrids) and are given in the upper part of the table. Performance in the 'forced-choice' maze is described by 3 statistics PL, CL and CR[3]. PL is the probability of turning left at the initial choice, as determined from the distribution of flies in the 8 collection tubes at the end of the maze. PR = 1-PL is the probability of going right initially. From the distribution of flies in the 4 left-hand tubes, the probability of going left after 5 forced turns can be determined. CL is the difference between this second probability and PL, while CR is the corresponding value for flies turning right. Clearly, if the probabilities do not change between the 2 choicepoints, CL and CR will both equal zero, while if the flies are more likely to repeat their initial response, then CL and CR will be positive. Performance at the third choice-point is not considered since it is known to be greatly influenced by the proximity of food in the collection tubes.

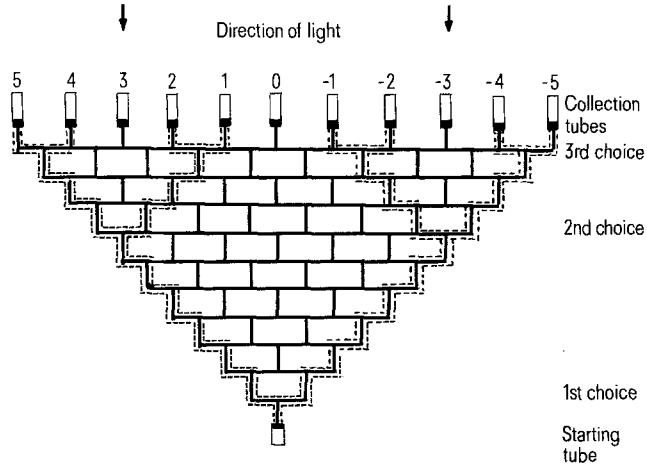
The F_1s have higher values of CL and CR than their parents. The significance of such differences can be judged from the parameter [h] which is the difference between the F_1 mean and m, the average of the parental strains[10]. Values for [h] were very similar in the LM 20×26 and 27×28, so those in the table have been averaged over both sets of crosses. The significant [h] values indicate a large amount of directional dominance or, more strictly, 'potence'[10], so that between 85 and 90% of all hybrids will repeat the initial response at the second choice-point.

Previously[3,8], when just strain differences were discussed, it was pointed out that significant values of CL and CR can only be attributed to learning, after eliminating alternatives such as right-left turning bias, wall-hugging, sequential alternation, sensitization, or other behaviours that may result in response repetition. In the present data, PL shows no dominance so that a turning bias cannot explain the dominance in CL and CR. Other possibilities are similarly excluded by the data in the last 2 columns of the table. These represent the proportion of flies ending up in the outer 2 left and right collection tubes of the maze where no passages were blocked off. These are the same tubes reached by flies that repeat the response in the 'forced-choice' maze, but now, where all alternatives are available, flies that reach the outer tubes would presumably do so as a result of 'wall-hugging' or similar behaviour. There is no dominance for increased numbers going to the outer tubes, which would be required for such a non-learning explanation of the CL and CR dominance in the other maze.

More detailed genetic analyses seem premature until ongoing experiments with a more flexible maze design[11] indicate the optimum behavioural conditions for learning to occur. However the intercross results do confirm a simple additive-dominance model for learning in the 'forced-choice' maze. The expected mean of this cross on such a model assuming no epistasis is the parental mean, m, $+\frac{1}{2}$[h], giving values of 27.4 for CL and 30.6 for CR, both of which are within 1% of the observed values in the table.

To confirm these results and assess the role of the light cues in the maze performance, the entire experiment was repeated in total darkness with a second set of 28,800 flies, the results being given in the lower half of the table. For the parental strains, CL and CR are now close to zero, indicating that without light cues these flies are less likely to repeat the response, that is, to demonstrate learning. Values for the hybrids are similarly reduced, although the significant [h] values show they are still more likely to learn than the parental strains. Apart from some reduction in the proportion of flies ending up in the left-hand tubes, the results in the other maze are very similar to these in the light, especially in the absence of any significant [h] values, which is to be expected if this maze only involves sequential alternation or 'wall-hugging', where kinaesthetic and other non-visual cues are important[3].

The directional dominance found for rodent learning[6] and



Schematic diagram of conventional maze (solid lines) and 'forced-choice' maze (dashed lines). The light source is a 10 W fluorescent lamp, the same width as the maze.

Performance of *Drosophila* crosses in 2 types of maze, with and without light cues

Genotype	With light cues					Without light cues				
	'Forced-choice' maze			Conventional maze		'Forced-choice' maze			Conventional maze	
	PL[a]	CL	CR	Outer left[b]	Outer right	PL[a]	CL	CR	Outer left[b]	Outer right
Parental strains[c]	51.5±1.3	22.9±2.1	23.8±3.7	22.2±1.7	11.1±0.8	47.7±1.5	6.8±2.6	0.3±2.7	12.9±1.3	12.9±1.8
Reciprocal F_1s[c]	52.1±1.4	32.0±2.7	37.5±1.8	19.3±1.5	13.5±1.2	47.5±1.9	15.2±1.9	13.2±2.4	15.4±1.1	13.3±1.2
Intercross[d]	54.9±2.0	27.7±3.7	30.5±2.8	19.8±1.8	15.3±1.4	43.0±2.3	12.5±2.6	14.6±2.7	17.4±1.4	15.4±2.1
[h][e]	0.6±2.1	9.1±3.5[g]	13.7±4.1[g]	−2.9±2.3	2.4±1.4	−0.2±2.4	8.4±3.2[f]	12.9±3.6[g]	2.5±1.7	0.4±2.1

[a] PL is the initial percentage of flies going left and CL and CR the changes in the percentage going left or right after 5 forced turns in that direction. [b] The percentages of flies that went to the 2 outermost tubes on the left or on the right, rather than to the central 7 tubes. [c] Each mean±SEM has 12 d.f., based on 3 d.f. within each of the 4 genotypes contributing to the mean. [d] Each mean±SEM has 14 d.f. with 7 d.f. from each of 2 genotypes. [e] The difference between the F_1 and parental mean [h], has 24 d.f.[10]. Values of [h] differing from zero on Student's t-test are indicated thus [f] $p<0.05$, [g] $p<0.01$.

possibly even for human intelligence[12] suggests these may be fitness characters with high performance conferring some advantage. It would be tempting to justify the present learning task in the same way, the sequence of turns required for learning being very similar to an insect's path towards a food odour[13]. However, the main points are that directional dominance has been found in *Drosophila*, paralleling that in rodents and that it distinguishes learning from the other behavioural components in the maze. It is reassuring that similar directional dominance has recently been observed in different strains of *D. melangogaster* with a very different learning task[2] involving electric shock[14].

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Y chromosome in the sibling species *Anopheles atroparvus* (van Thiel, 1927) and *A. labranchiae* (Falleroni, 1926) (Diptera: Culicidae): differential behaviour of the short arm after acid-alkaline treatment and Coriphosphine-O staining

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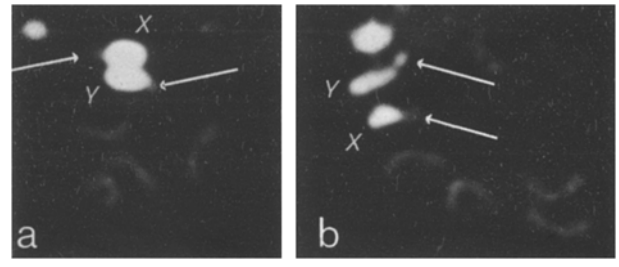
Summary. An acid-alkaline treatment followed by Coriphosphine-O staining was used for detecting chromosomal differences between the 2 sibling species *Anopheles atroparvus* (van Thiel) and *A. labranchiae* (Falleroni) (Diptera: Culicidae). The short arm of the Y chromosome was found to stain differently in the 2 species.

The paleartic *Anopheles maculipennis* complex includes 6 species of which 2, *Anopheles atroparvus* and *A. labranchiae*, are considered the most closely related genetically[2,3]. All these species show an identical karyotype (2n = 6) which consists of 2 pairs of metacentric autosomes and 1 pair of submetacentric sex chromosomes. Ethological and ecological, in addition to cytogenetical and biochemical, investigations were carried out for distinguishing the above-mentioned 6 species[4–7]. The hybridological approach, moreover, either without or in connection with the observation of polytene banding patterns, has been used for detecting specific differences, particularly between *A. atroparvus* and *A. labranchiae*[5,8,9].

The only cytological differences between these 2 species were found with Q-banding or with the labelling of replicating DNA in the hybrid polytene chromosomes[10,11]. Such differences, however, involve the whole chromosomal complement on the one hand (Q-banding), or asynaptic polytene regions on both heterosomes and autosomes on the other hand (differential DNA synthesis). The Y chromosome was never found to show specific differences between the 2 species considered, which, moreover, show complete homology in karyotype[10], DNA replication in metaphase chromosomes (Fraccaro et al.[12], and Marchi, unpublished personal observations), and polytene banding pattern[13].

Cytotaxonomic markers are now available, which can complement the banding pattern of polytene chromosomes. A number of techniques make possible a linear differentiation of metaphase chromosomes in several organisms, including Diptera[12,14,15]. We used an acid-alkaline treatment followed by Coriphosphine-O staining for differentiating metaphase chromosomes of *A. atroparvus* and *A. labranchiae*. The possible correlations between these cytological results and previous hybridological and genetical data will be discussed.

Materials and methods. A. atroparvus larvae were provided by the Genetics Institute of this University, through the courtesy of Prof. G. Frizzi, while *A. labranchiae* were collected in the field from several areas of Sardinia. Brains and/or gonads of 4th instar larvae of both sexes were dissected in a modified Ringer's solution (NaCl 0.9 g, KCl 0.42 g, $CaCl_2$ 0.25 g in 1000 ml of distilled water). After fixation in methanol: acetic acid (3:1), tissues were incubated in 45% acetic acid for 2 min. After squashing, slides were kept in solid CO_2 for 5 min and the siliconized coverslips were removed. Air-dried preparations were left to age for 5–7 days at 21 ∓ 2 °C and then processed as follows: a) immersion in 1 N HCl at 21 ∓ 2 °C for 10 min and rinsing in distilled water; b) bath in 5% $Ba(OH)_2 \cdot 8\ H_2O$ at 52 °C for 4 min and washing in distilled water; c) staining with Coriphosphine-O according to Keeble and Jay[16]. Control slides were stained with the same fluorochrome, either



Metaphase chromosomes of *A. atroparvus* (a) and *A. labranchiae* (b) after acid-alkaline treatment and Coriphosphine-O staining. *a A. atroparvus:* the arrows indicate the short arms of the sex chromosomes. The long arms reveal close pairing and bright fluorescence. The 2 autosome pairs are located under X and Y and show low fluorescent intensity like the short arms of sex chromosomes. *b A. labranchiae:* the short arms reveal a different fluorescent staining in sex chromosomes, being bright on the Y and dull like the autosomes on the X (see arrows).