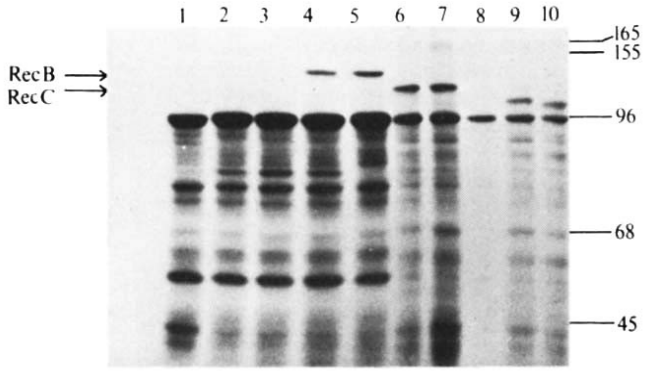

Fig. 4 ^{35}S-methionine labelled proteins produced in maxicells by the plasmids pPE230 (*recB*$^+$) and pPE220 (*recC*$^+$) and their derivatives in which $\gamma\delta$ is inserted in the *recB* or *recC* gene ($\gamma\delta$1–3) or in a gene other than *recB* or *recC* ($\gamma\delta$0), Tracks: 1, pPE230-$\gamma\delta$3; 2, pPE230-$\gamma\delta$2; 3, pPE230-$\gamma\delta$1; 4, pPE230-$\gamma\delta$0 (*recB*$^+$); 5, pPE230 (recB$^+$); 6, pPE220 (*recC*$^+$); 7, pPE220-$\gamma\delta$0 (*recC*$^+$); 8, pPE220-$\gamma\delta$1; 9, pPE220-$\gamma\delta$2; 10, pPE220-$\gamma\delta$3. Proteins were separated by electrophoresis on an 8% polyacrylamide gel and visualized by fluorography. Molecular weights, determined by reference to unlabelled protein standards followed by staining, are indicated. The standards were RNA polymerase β' (165,000), RNA polymerase β (155,000), phosphorylase *b* (96,000), bovine serum albumin (68,000) and ovalbumin (45,000).

preparations of the RecBC enzyme that were 90% pure were the *recB* and *recC* products, respectively.

Our cloning of the *recB* and *recC* genes individually into a multicopy plasmid may lead to an easier method of isolating the individual subunits of the RecBC enzyme and should permit investigation of their partial properties. Our results do not rule out the possibility that active RecBC DNase includes one or more subunit(s) encoded by a gene or genes other than *recB* and *recC*. However, purification of the individual RecB and RecC subunits will permit studies of the reconstituted enzyme to help answer this question. Recent results show that at physiological ATP concentrations and in the presence of Ca^{2+} the RecBC DNase uses energy derived from ATP hydrolysis to travel through duplex DNA, unwinding the DNA ahead of itself and rewinding it behind[15–17]. In this regard, the RecBC DNase resembles other enzymes such as the helicases, gyrase and RecA protein which use the energy derived from ATP hydrolysis to modify the physical structure of DNA. Cloning of the *recA* gene[18–20] and the resultant ease with which the RecA protein could be isolated paved the way for the biochemical experiments which have greatly helped to explain the role of the RecA protein in genetic recombination and DNA repair[21–23]. The cloned *recB* and *recC* genes may also facilitate the isolation of the RecBC DNase and stimulate further experiments to determine its role in the cell.

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Sexual activity reduces lifespan of male fruitflies

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Many theories on the evolution of life histories have assumed a physiological cost of reproduction in terms of reduced lifespan[1–3]. A cost of increased reproduction in terms of reduced longevity has been established experimentally for females, both as an additive genetic[4,5] and as a purely phenotypic[6,7] effect. Such a physiological cost of reproduction has not been demonstrated for males. The cost of sexual activity has been assumed to be relatively small in those species where the only paternal contribution to an offspring is the gamete[8,9]. Here we show that increasing sexual activity reduces longevity in the male fruitfly (*Drosophila melanogaster*) and hence that there is a significant physiological cost of male sexual activity in a species where the father contributes only gametes to his progeny.

The flies used were an outbred stock collected in Dahomey in 1970. Sexual activity was manipulated by supplying individual males with receptive virgin females at a rate of one or eight virgins per day. The longevity of these males was recorded and compared with that of two control types. The first control consisted of two sets of individual males kept with newly inseminated females equal in number to the virgin females supplied to the experimental males. Newly inseminated females will not usually re-mate for at least 2 days[10,11] thus they served as a control for any effect of competition with the male for food or space. The second control was a set of individual males kept with no females. There were 25 males in each of the experimental and control groups, and the groups were treated identically in respect of number of anaesthetizations (using CO_2) and provision of fresh food medium.

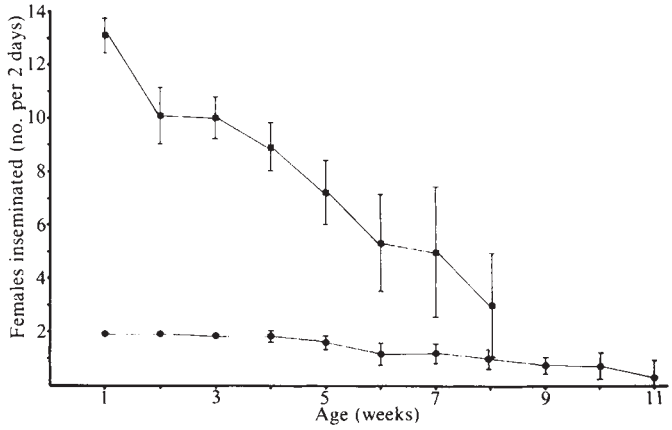

Fig. 1 The relationship between insemination rate (number of females inseminated per 2 days) and age (weeks) for males kept with one virgin female (●) or eight virgin females (■) per day. Error bars are 95% confidence limits.

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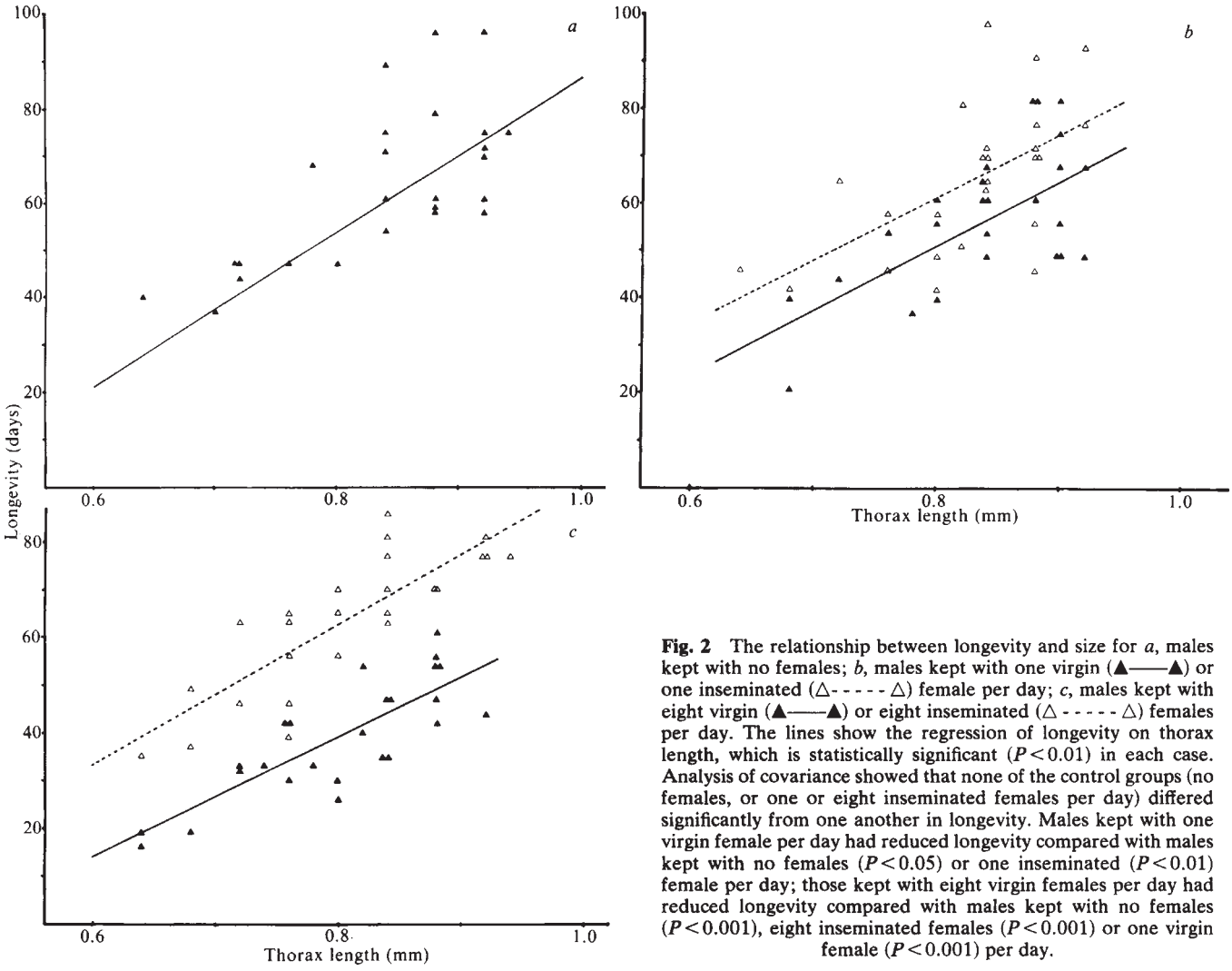

Fig. 2 The relationship between longevity and size for *a*, males kept with no females; *b*, males kept with one virgin (▲——▲) or one inseminated (△- - - - -△) female per day; *c*, males kept with eight virgin (▲——▲) or eight inseminated (△ - - - - -△) females per day. The lines show the regression of longevity on thorax length, which is statistically significant ($P<0.01$) in each case. Analysis of covariance showed that none of the control groups (no females, or one or eight inseminated females per day) differed significantly from one another in longevity. Males kept with one virgin female per day had reduced longevity compared with males kept with no females ($P<0.05$) or one inseminated ($P<0.01$) female per day; those kept with eight virgin females per day had reduced longevity compared with males kept with no females ($P<0.001$), eight inseminated females ($P<0.001$) or one virgin female ($P<0.001$) per day.

On two days per week throughout the life of each experimental male, the females that had been supplied as virgins to that male were kept and examined for fertile eggs. This gave an estimate of the insemination rate of the two groups of males (Fig. 1). In both groups the insemination rate declined with the age of the male, and the rate was higher for males supplied with eight virgins per day than for those supplied with only one virgin per day. There were no significant differences in insemination rate between the individual males within each experimental group.

In the absence of any sexual activity, the longevity of male fruitflies is associated with their size (Fig. 2*a*). Size was therefore taken into account when examining the effect of sexual activity on longevity (Fig. 2*b*, *c*). Analysis of covariance showed that: (1) there were no significant differences in longevity between the control groups (median longevity 65 days); (2) the males supplied with one virgin female per day had significantly reduced longevity (median 56 days) compared with males in any control group; (3) males kept with eight virgin females per day had significantly reduced longevity (median 40 days) compared with males kept with one virgin female per day, and control males. These results show that male sexual activity reduces longevity, and that this effect is more marked for a higher level of sexual activity.

Physiological costs of particular activities have generally been discussed in terms of the diversion of nutrients into these activities at the expense of others[12]. In our experiment, energetic costs of sexual activity would have included the production of sperm and seminal fluid and the muscular action associated with mating itself. In addition to inseminating females, the experimental males probably also performed higher levels of courtship than control males. The control males kept without females performed no courtship. When inseminated females are courted they extrude the ovipositor, which terminates the male courtship[13]. In nature, nutritional effects may be increased by food shortage, and there may be costs of sexual activity in addition to those detected here. Searching for mates[14] and fighting with other males[15] may be costly physiologically and these activities, together with courtship and mating, may make males more vulnerable to predation[16].

Sexual activity could affect longevity in two ways. First, it may have an effect on the probability of death occurring in the next short period of time. Cessation of sexual activity at any age would then leave a fly with a life expectancy comparable with that of controls of the same age. High temperatures can have such an effect on longevity in *Drosophila subobscura*[17]. Second, sexual activity may have some cumulative, possibly irreversible, effect. Williams[18] has suggested that senescence may be caused by the deleterious pleiotropic effects later in life of genes which have beneficial effects early in life. A deleterious long-term effect of sexual activity earlier in life could produce such a pleiotropic effect. Phenotypic correlations of the kind found in these experiments need not necessarily mean that a genetically caused change in the level of male sexual activity would alter longevity. A negative additive genetic correlation would be needed to demonstrate this and should be the subject of further experiments.

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Pulmonary vein as an ectopic focus in digitalis-induced arrhythmia

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The tunica media of the pulmonary vein (PV) in many mammalian species, including man, is made up of cardiac muscle[1–3]. Brunton and Fayrer first reported that independent pulsation of the PV occurred in cats and rabbits even after activity of the heart had ceased[4]. Electrophysiological studies have also shown spontaneous electrical activity in isolated PVs of the guinea pig and a pacemaking region has been located at the distal end of the cardiac portion of the vein adjoining the smooth muscle. The intrinsic frequency of the PV was low and activity at the PV was normally coupled to the sinus rhythm[5]. Thus, the PV behaves as a subsidiary pacemaker. In the presence of digitalis-type agents, subsidiary pacemakers such as Purkinje fibres develop oscillatory afterpotentials (OAPs) which may be large enough to reach threshold[6,7], and it has been suggested that OAPs at Purkinje fibres leading to spontaneous action potentials may provide the underlying mechanism for ventricular arrhythmia during digitalis intoxication[6]. In contrast, atrial and ventricular muscles are not very sensitive to the arrhythmogenic action of digitalis[8]. I have therefore now investigated whether the PV can develop OAPs and act as an ectopic focus with digitalis intoxication. By recording with intracellular microelectrodes simultaneously at the PV and right atrium, I demonstrate that OAPs and repetitive activity develop at the PV in the presence of ouabain. Propagation of these triggered action potentials at the PV into the atrium results in atrial extrasystoles.

The recording arrangements in these experiments were similar to those reported previously[5]. Using male guinea pigs (300–400 g), intracellular recordings were made with glass micropipettes filled with 3 M KCl. All recordings were made at the distal end of the cardiac PV and the dorsal surface of the intact right atrium close to the vena cava. The experiments were carried out at 32–34 °C, the preparations being superfused continuously with normal or ouabain-containing physiological solutions at a rate of 4 ml min^{-1}.

In spontaneously active preparations, the PV was driven by impulses originating from the SA node[5]. In the presence of ouabain (0.5–1.0 μM), the sinus rate decreased and became less regular, and in all seven preparations studied, after 30–40 min OAPs developed at the PV but not in atrial muscle cells (Fig. 1). Diastolic potentials between action potentials remained flat at the atrial recording site.

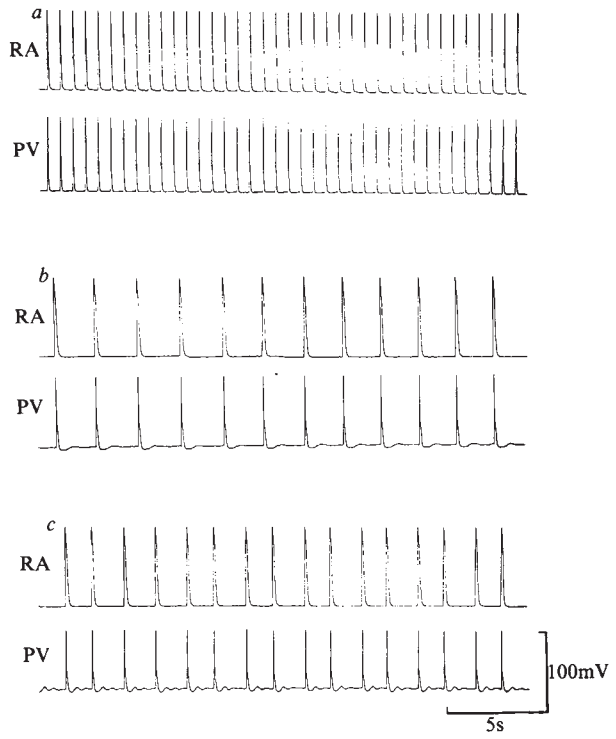

Fig. 1 Effect of ouabain (0.5 μM) on the electrical activity of the pulmonary vein (PV) and the right atrium (RA). As demonstrated previously, action potentials at the PV are closely coupled to those at the RA[5]. After exposure to ouabain for 40 min, OAPs developed at the PV (*b*) and became very prominent at 50 min (*c*). No membrane oscillation was observed at the RA. The spontaneous frequency of the preparation decreased in the presence of ouabain.

When higher ouabain concentrations were used (≥2 μM), high frequency repetitive activity appeared in six out of six preparations. Figure 2 demonstrates the onset of repetitive activity in a preparation after exposure to ouabain (2.5 μM) for 75 min. Initially, two short bursts of extrasystoles were observed (Fig. 2*a*), soon followed by a period of repetitive activity lasting for about $2\frac{1}{2}$ min at both the PV and the right atrium. Another train of repetitive activity followed after a brief pause (Fig. 2*b*).

Closer examination of the record revealed that the extrasystoles were of PV origin triggered by the sinus beats. In normal conditions, action potentials at the right atrium always preceded those at the PV[5]. The first pair of action potentials of each train of repetitive activity was also led by the atrial muscle, indicating the sinus origin of these action potentials. However, the lead was shifted to the PV in all subsequent action potentials of the train, suggesting the diversion of the pacemaking site to a region close to the PV (Fig. 2*c*). These action potentials at the PV were characterized by large diastolic depolarizations. In contrast, action potentials at the right atrium arose abruptly from the baseline membrane potential. Subthreshold OAPs were observed at the PV but not at atrial sites after termination of each train. Figure 2*d* also demonstrates the initiation of a second train of repetitive activity triggered by the sinus beat and the subsequent shift of pacemaking site to the PV.

The amplitude of action potentials at the PV decreased gradually with time in ouabain. This decrease was more marked during repetitive activity. For example, the amplitude of action potentials decreased by about 30 mV at the end of the long train in Fig. 2*b* and remained small subsequently. There was also a slight decrement in the amplitude of action potentials at the right atrium during repetitive activity.

The duration of each period of repetitive activity decreased drastically within 10 to 20 min after their appearance. For example, 5 min after the long train shown in Fig. 2*b*, only three action potentials appeared in each train (Fig. 3*a*). This further

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