ticular flow. Indeed, specimens heated to higher temperatures seem to lose much of their ability to acquire palaeo-TRM, when compared with specimens heated to temperatures below 300 °C. We suspect that as the peak temperature approaches 300 °C, an increasing fraction of magnetic grains alter in such a manner that their blocking temperatures are raised significantly. Thus, those grains responsible for the acquisition of laboratory palaeo-TRM at lower temperatures during the (second) field-cooling may lose this capability just before being cooled from a higher temperature.

Adopting this as a working hypothesis enables us to explain the significantly steepened, single-segment Thellier result (Fig. 1*c*): although the thermal treatment of subsamples employed in the multi-specimen determination may not be sufficient to produce detectable physicochemical alteration until the assigned temperature approaches 300 °C, the cumulative effect of stepwise heatings associated with the Thellier method may indeed cause significant sample degradation which is observable at lower temperatures. A single-segment, spuriously steepened plot may be roughly explained if we take as our starting point the multi-specimen data (Fig. 3) and apply with each successive temperature step up to 300 °C a progressively reduced intensity of blocked-in lab-TRM.

Thus it seems that the true palaeointensity for basalt flow 1 is better determined from the slope of the upper (that is, lower-temperature) segment of Fig. 3, that is, from data associated with specimens that are least likely to have been altered during laboratory heating. A palaeofield strength $H_P \approx 100\ \mu T$ is determined from this reasonably well defined linear segment, which is only 30% of the Thellier result.

In contrast to flow 1, the application of both methods to the adjacent flow (flow 2) gives single, linear segments over a considerable range of temperature (Fig. 4). Subjected to the same laboratory field strength, $H_L = 170\ \mu T$, the slopes are found to be essentially identical (associated with a palaeointensity $H_P = 45\ \mu T$). Clearly, the specimen degradation which occurred during thermal treatment of flow 1 did not occur during these determinations. Moreover, because of the very different heating procedures involved with the two methods, we argue that physicochemical alteration may be confidently ruled out as a source of error and that the palaeointensity determination for flow 2 is very reliable.

Thus we obtained reliable palaeointensity determinations for both basalt flows (1 and 2) by using the multi-specimen method. Clearly, the Thellier approach fails for the flow 1 samples considered here, even though the palaeointensity data, and other relevant rock-magnetic properties, give the impression of credibility. Why such 'ideal' basaltic rocks are apparently incapable of providing reliable Thellier results is now under investigation. Continued testing and use of the new method will ultimately determine the extent of its utility to archaeomagnetic and palaeomagnetic studies. □

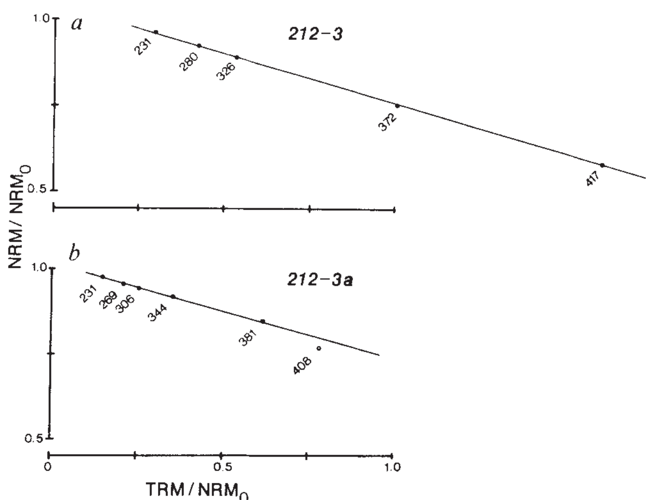

FIG. 4 Determinations by *a*, the multi-specimen and *b*, the Thellier methods, for flow 2 (site 212), employing the same H_L (=170 μT). The palaeointensity H_P=45 μT is the same for both determinations. (Note that the Thellier data show signs of alteration effects above 400 °C, but the multi-specimen data do not.)

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Oxidation of aromatic contaminants coupled to microbial iron reduction

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THE contamination of sub-surface water supplies with aromatic compounds is a significant environmental concern[1,2]. As these contaminated sub-surface environments are generally anaerobic, the microbial oxidation of aromatic compounds coupled to nitrate reduction, sulphate reduction and methane production has been studied intensively[1-7]. In addition, geochemical evidence suggests that Fe(III) can be an important electron acceptor for the oxidation of aromatic compounds in anaerobic groundwater. Until now, only abiological mechanisms for the oxidation of aromatic compounds with Fe(III) have been reported[8-12]. Here we show that in aquatic sediments, microbial activity is necessary for the oxidation of model aromatic compounds coupled to Fe(III) reduction. Furthermore, a pure culture of the Fe(III)-reducing bacterium GS-15 can obtain energy for growth by oxidizing benzoate, toluene, phenol or *p*-cresol with Fe(III) as the sole electron acceptor. These results extend the known physiological capabilities of Fe(III)-reducing organisms and provide the first example of an organism of any type which can oxidize an aromatic hydrocarbon anaerobically.

At the onset of anaerobic conditions, Fe(III) is the most abundant potential electron acceptor for organic-matter oxidation in most fresh-water sedimentary environments[13,14]. The accumulation of Fe(II) during the anaerobic oxidation of organic matter in numerous pristine and contaminated aquifers further suggests that Fe(III) is a potential electron acceptor in a wide variety of sub-surface environments[15,16]. An example of the potential role of Fe(III) in oxidizing contaminants in groundwater was observed in a glacial-outwash aquifer located in Bemidji, Minnesota. The aquifer was contaminated by crude oil from a break in a pipeline in 1979[17]. A plume of anaerobic groundwater developed down gradient from the oil body that floats on the water table. Benzene and alkylbenzenes are leaching

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into the anaerobic groundwater but their concentrations decrease rapidly along the groundwater flow path[18,19]. Isomeric alkylbenzenes decrease in concentration at different rates in the anaerobic plume, indicating that the aromatic contaminants are not removed by physical-chemical processes such as adsorption, volatilization or dilution[18,19]. In 1984, initial studies on the anaerobic groundwater at a site, Bemidji A, near the edge of the oil lens indicated that the dissolved inorganic carbon in the contaminated groundwater was isotopically lighter than that in the native groundwater (Fig. 1). This suggested that the isotopically light contaminant hydrocarbons ($\delta^{13}C$ of −28‰) were being converted to carbon dioxide.

Fe(III) appeared to be an important electron acceptor for the anaerobic oxidation of the aromatic hydrocarbons to carbon dioxide. Fe(II), the reduced product of the reduction of Fe(III), accumulated over time in the contaminated groundwater at Bemidji A but was undetectable in the native groundwater at an uncontaminated background site (Fig. 1). By 1988 cores of sediments from Bemidji A contained only 4 mmol of oxalate-extractable Fe(III) (ref. 20) per kg of sediment. Sediments from the uncontaminated background site contained four times as much Fe(III). The native groundwater contained too little nitrate or sulphate (less than 20 μM, as determined by ion chromatography) for these electron acceptors to be significant anaerobic oxidants of the contaminants.

By 1987 it appeared that methane production had also become a terminal electron-accepting process at Bemidji A (Fig. 1). This transition from Fe(III) reduction as the predominant terminal electron-accepting process to simultaneous Fe(III) reduction and methane production is typically observed in fresh-water sediments as the availability of Fe(III) for microbial reduction diminishes[21,22]. Although the inorganic carbon produced during this period was still isotopically lighter than that in the native groundwater, it became progressively heavier, presumably as a result of methanogenic bacteria preferentially reducing light carbon dioxide to methane[23].

The increasing importance of methane production at Bemidji A meant that studies on the oxidation of aromatic compounds with Fe(III) as the only electron acceptor were no longer possible at this site. Thus, in 1988 a new site, designated Bemidji B, was sampled with a split-spoon corer to obtain sub-surface sediments in which Fe(III) reduction was still the predominant terminal electron-accepting process. Bemidji B, which was located only 5 m further along the flow path of the anaerobic plume than Bemidji A, was still found to contain ample oxalate-extractable Fe(III), (17 mmol per kg of sediment), to support Fe(III) reduction. Using strict anaerobic techniques[24], Bemidji B sediments (20 ml) were transferred under N_2-CO_2 (at a ratio of 93:7) into serum bottles (30 ml capacity) which were then sealed with butyl rubber stoppers. Fe(III) reduction and methane production were monitored as previously described[24]. After a two-month incubation at the *in situ* temperature of 9 °C, 1.5 mmol of HCl-extractable Fe(II)[24] had accumulated per kg of sediment and no methane had been produced. This result indicated that Fe(III) reduction was the predominant terminal electron-accepting process for organic-matter oxidation in the sediments collected from Bemidji B.

To determine whether the apparent oxidation of aromatic hydrocarbons to carbon dioxide during the reduction of Fe(III) in sub-surface sediments was the result of abiotic or biotic reactions, Bemidji B sediments (25 ml) were anaerobically placed into serum bottles (50 ml) which were then completely filled with anaerobic groundwater from Bemidji B. The bottles were sealed without a headspace with Teflon-lined butyl rubber stoppers. Toluene was added with a microsyringe to provide an initial concentration of 600 μM. Toluene concentrations were measured by extracting the sediment slurries with pentane and quantifying the toluene in the extract by flame ionization-gas chromatography. After 45 days of incubation, less than 2% of the toluene remained. However, in sediments in which the microorganisms had been killed with heat (121 °C for 1 h) before incubation, there was no loss of toluene and no Fe(III) reduction. These results suggest that the disappearance of toluene during the reduction of Fe(III) was microbially mediated.

To examine further the potential role of microorganisms in the oxidation of aromatic compounds coupled to the reduction of Fe(III), the oxidation of benzoate in the Fe(III)-reducing sediments was investigated. Benzoate is most frequently used as a model compound for aromatic decomposition studies[3] and the accumulation of benzoate and methylbenzoate compounds in the contaminated groundwaters of the Bemidji aquifer suggest that benzoate is an important intermediate in the oxidation of monoaromatic hydrocarons under Fe(III)-reducing conditions[19]. When the Bemidji B sediments in which Fe(III) reduction was the terminal electron-accepting process were injected with uniformly ring-labelled [^{14}C]benzoate under anaerobic

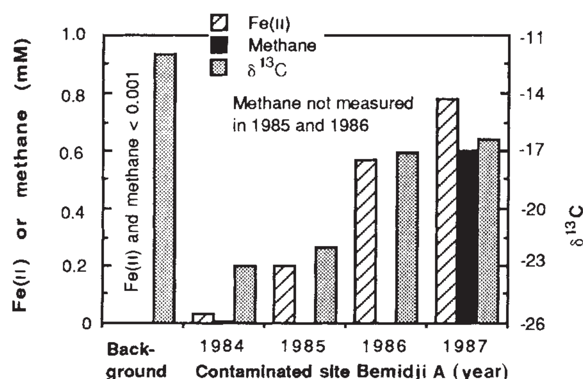

FIG. 1 Concentrations of Fe(II) and methane and the $\delta^{13}C$ (relative to the PDB standard) of the inorganic carbon dissolved in the anaerobic groundwater at site Bemidji A, and in the aerobic native groundwater at a nearby uncontaminated background site. Groundwater was sampled with a peristaltic pump and dissolved Fe(II) and methane were determined with standard geochemical techniques as previously described[23].

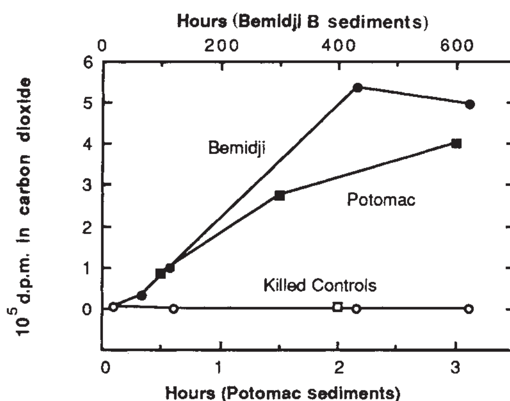

FIG. 2 Production of [^{14}C]-carbon dioxide from uniformly ring-labelled [^{14}C]-benzoate in sediments with Fe(III) reduction as the terminal electron-accepting process. Bemidji sub-surface sediments were from site Bemidji B, and Potomac River sediments were surface fresh-water sediments in which it was known[22] that Fe(III) reduction was the terminal electron-accepting process. [^{14}C]benzoate (0.1 ml containing 0.35 μCi of 120 mCi per mmol benzoate) was anaerobically added to 10 ml of sediment that was incubated under N_2-CO_2(in a ratio of 93:7) in anaerobic pressure tubes which were sealed with butyl rubber stoppers. At the designated times 1 ml of 2.5 M H_2SO_4 was added with a syringe and needle. N_2 was flushed through the tubes and into a series of vials containing 1 M NaOH to trap $^{14}CO_2$. A scintillation counter was used to determine $^{14}CO_2$. For Bemidji sediments, killed controls were treated with heat (121 °C for 1 h) before the addition of [^{14}C]benzoate. For Potomac river sediments, killed controls received additions of glutyraldehyde (2.5% final concentration) before the addition of [^{14}C]benzoate.

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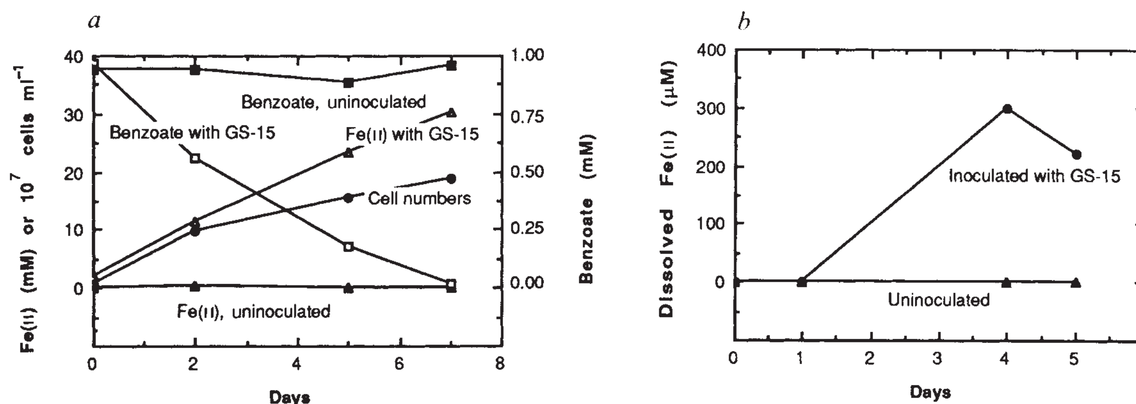


FIG. 3 Benzoate metabolism coupled to reduction of Fe(III) by the dissimilatory Fe(III)-reducing bacterium, GS-15. *a*, Growth in a medium[28] with benzoate as the sole electron donor and Fe(III)-citrate as the sole electron acceptor. Benzoate was determined with HPLC. Fe(II) and cell numbers were quantified with ferrozine and by microscopy, respectively as previously described[28]. *b*, Production of dissolved Fe(II) when subsurface sediments (10 ml) from an uncontaminated portion of the Bemidji aquifer were mixed with an equal volume of medium[28] with 1.5 mM benzoate as the sole electron donor. The Fe(III) in the sediments was the only electron acceptor provided.

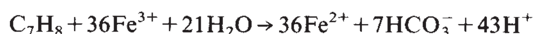
conditions, the [^{14}C]benzoate was oxidized to $^{14}CO_2$, but only if biological activity was not inhibited (Fig. 2). A similar biologically dependent oxidation of [^{14}C]benzoate was observed in fresh-water sediments from the Potomac River in which Fe(III) reduction was the predominant terminal electron-accepting process (Fig. 2).

It has long been recognized that microbial activity can enhance the oxidation of organic compounds coupled to the reduction of Fe(III)[25,26]. However, the ability of Fe(III)-reducing microorganisms to directly link the complete oxidation of organic compounds to Fe(III) reduction has only recently been recognized[27-30]. The dissimilatory iron-reducing bacterium, strain GS-15, was previously known to obtain energy for growth by oxidizing short-chain fatty acids or ethanol to carbon dioxide with amorphous Fe(III) oxide or Fe(III) citrate as the sole electron acceptor[28]. However, after an adaptation period (~1 month) GS-15 grew vigorously with benzoate and either of the Fe(III) forms. Growth was associated with the metabolism of benzoate and the concomitant reduction of Fe(III) to Fe(II) (Fig. 3*a*). The stoichiometry of benzoate metabolism (Fig. 3*a*) was consistent with the reaction

$$C_6H_5COO^- + 30Fe^{3+} + 19H_2O \rightarrow 30Fe^{2+} + 7HCO_3^- + 36H^+$$

With benzoate as the sole electron donor, GS-15 could also reduce the Fe(III) in the sub-surface sediments at Bemidji (Fig. 3*b*). This demonstrates that there are microorganisms that can couple the oxidation of aromatic compounds to the reduction of naturally occurring Fe(III) oxides.

GS-15 also reduced Fe(III) in a medium[28] with toluene as the sole electron donor and amorphous Fe(III) oxide as the sole electron acceptor. The volatility of toluene and its tendency to be absorbed into the butyl rubber stoppers prevented quantitative measurements of toluene uptake. However, carbon dioxide production and Fe(III) reduction were measured as previously described[28] after GS-15 was cultured through three successive transfers (10% inoculum) on toluene–Fe(III) medium. In a medium containing no more than 1 mmol l^{-1} toluene as the sole electron donor, and amorphous Fe(III) oxide as the sole electron acceptor, GS-15 produced 1.6 mM carbon dioxide and 9.3 mM Fe(II) during a two week incubation. No extracellular intermediates were detectable with high-performance liquid chromatography. These results are consistent with the reaction



$$C_7H_8 + 36Fe^{3+} + 21H_2O \rightarrow 36Fe^{2+} + 7HCO_3^- + 43H^+$$

Although the oxidation of aromatic hydrocarbons has recently been documented in anaerobic systems containing mixed microbial populations with nitrate reduction or methane production as the terminal electron-accepting process[2,5-7], GS-15 is the first organism in pure culture known to oxidize an aromatic hydrocarbon under anaerobic conditions.

GS-15 was also capable of oxidizing two other common aromatic contaminants, phenol and *p*-cresol, at initial concentrations of 1 mM, with amorphous Fe(III) oxide as the electron acceptor. GS-15 could not oxidize other model aromatic compounds like phthalic acid, nicotinic acid, ferulic acid or syringic acid. However, enrichment cultures which oxidize these compounds with the reduction of Fe(III) were readily obtained by inoculating sediments into an anaerobic medium (pH 6.7) (ref. 28) that contained one of the aromatics as the sole electron donor and amorphous Fe(III) oxide as the sole electron acceptor. None of the aromatic compounds metabolized by GS-15, or the enrichment cultures reduced Fe(III), in the absence of microorganisms. In testing a wide variety of aromatic compounds we have found only one, catechol, which abiologically reduces amorphous Fe(III) oxide at the near-neutral pH values typical of most groundwater environments. Given the widespread availability of Fe(III) as a potential electron acceptor in subsurface environments[13-16], it seems likely that microbial oxidation of aromatic contaminants, coupled to dissimilatory Fe(III) reduction, may be capable of removing significant quantities of aromatic compounds from many contaminated aquifers. □

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Genetic segregation and the maintenance of sexual reproduction

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SEXUAL reproduction confronts evolutionary biology with a paradox: other things being equal, an asexual (all-female) population will have twice the reproductive potential of a competing sexual population and therefore should rapidly drive the sexual population to extinction[1,2]. Thus, the persistence of sexual reproduction in most life forms implies a compensatory advantage to sexual reproduction. Work on this problem has emphasized the evolutionary advantages produced by the genetic recombination that accompanies sexual reproduction[1–7]. Here we show that genetic segregation produces an advantage to sexual reproduction even in the absence of an advantage from recombination. Segregation in a diploid sexual population allows selection to carry a single advantageous mutation to a homozygous state, whereas two separate mutations are required in a parthenogenetic population. The complete fixation of advantageous mutations is thus delayed in a heterozygous state in asexual populations. Calculation of the selective load incurred suggests that it may offset the intrinsic twofold reproductive advantage of asexual reproduction and maintain sexual reproduction in diploid populations.

To determine the magnitude of the genetic load in an asexual population caused by loci caught in the heterozygous state, consider the fate of advantageous mutations arising at L homozygous wild-type loci, assuming for simplicity that L is constant. Let the mutant heterozygotes be intermediate between the wild-type and mutant homozygotes with fitness $1+hs$, 1 and $1+s$ respectively, where s and h are positive and <1. We also assume that no more than one advantageous mutation will be spreading through the population at any time to ensure that there is no advantage to sexual reproduction derived from recombination.

The probabilities that equivalent advantageous mutations at homozygous wild-type loci spread in an asexual and in a sexual population are approximately equal as segregation does not alter the relative fitnesses of the wild-type homozygote and the mutant heterozygote. If a mutation spreads, however, it will reach fixation for the homozygote in a sexual population via segregation but only fixation for the heterozygote in an asexual population (Fig. 1). The rate at which loci become fixed in the heterozygous state in an asexual population is $2\mu NLP_0$, where μ is the per allele mutation rate to advantageous alleles, N the population size and P_0 the probability that a single advantageous mutation at a homozygous wild-type locus spreads. To be converted completely to the advantageous homozygote, a second mutation must occur in a heterozygote and then spread. The rate at which loci fixed as heterozygotes are converted to the advantageous homozygotes in an asexual population is μNnP_1, where P_1 is the probability that a mutation converting a heterozygote to an advantageous homozygote spreads through the population and n is the number of loci fixed in the heterozygous state. Thus, the rate of change in the number of loci fixed in the heterozygous state in an asexual population is $dn/dt = 2\mu NLP_0 - \mu NnP_1$. From classical theory (ref. 3), $P_0 \approx 2hs$ and $P_1 \approx 2s(1-h)/(1+hs)$ when $s \ll 1$ and $Ns \gg 1$. At equilibrium, therefore, the number of loci in this state is

$$\hat{n} \approx \frac{2Lh(1+hs)}{1-h} \qquad (1)$$

These heterozygous loci create a genetic load for an asexual population because equivalent loci go on to fixation for the advantageous homozygote in a sexual population (Fig. 1). To calculate the magnitude of fitness loss for an asexual population we assume that the fitness effects of loci are simply multiplicative. The effect of this genetic load is to give a sexual population a fitness of

$$W_s = \left(\frac{1+s}{1+hs}\right)^n \qquad (2)$$

relative to an equivalent asexual population. When $W_s > 2$, this advantage to the sexual population more than compensates for its intrinsic twofold reproductive deficit.

Equilibrium values of W_s as a function of s and L are shown in Table 1. Equation (2) and Table 1 show that larger values of s and L produce increases in the equilibrium value of W_s and so give an increasing advantage to sexual reproduction. Even moderate selection coefficients and numbers of mutable loci are sufficient to maintain sexual reproduction (Table 1). The equilibrium for W_s is unaffected by the mutation rate μ or the population size N, but the rate at which loci fixed in the heterozy-

TABLE 1 Equilibrium values of W_s, the fitness of a sexual population relative to an asexual population

s \ L	10	50	100	500	1,000
0.001	1.0	1.1	1.1	1.6	2.7
0.005	1.1	1.3	1.6	1.2×10	1.5×10^2
0.01	1.1	1.6	2.7	1.5×10^2	2.1×10^4
0.05	1.6	1.2×10	1.4×10^2	5.3×10^{10}	2.8×10^{21}
0.1	2.7	1.3×10^2	1.7×10^4	1.6×10^{21}	2.7×10^{42}

Values below the heavy line exceed 2, indicating a net fitness advantage to the sexual population. Calculations assume $h=1/2$.

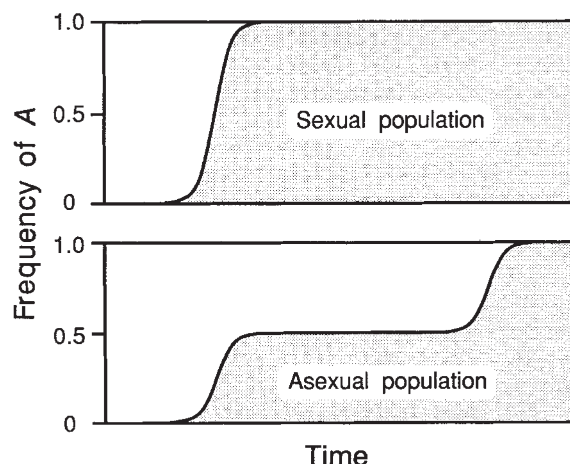

FIG. 1 Schematic illustration of the fates of an advantageous mutation *A* spreading through sexual and asexual diploid populations. The locus in the asexual population is fixed in a heterozygous state until a mutation appears at the second allele. The second mutation takes on average twice as long to appear, as only one allele is available to mutate in the heterozygote.

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