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# Occurrence in Wild Strains of *Neurospora crassa* of Genes Controlling Genetic Recombination

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*Abstract*

Each of the main laboratory wild stocks of *N. crassa* carries one of two alleles at the *rec-1* and *rec-2* loci and one of three at the *rec-3* locus. The constitutions of the stocks are given in Fig. 1. Some of those conserved are evidently not the originals. The third *rec-3* gene (*rec-3*$^{L}$), found in Lindegren A, controls recombination at the *am-1* locus to a level between that of *rec-3*$^{+}$ and *rec-3*, the relative levels being 1 : 8 : 25. At the *his-2* locus *rec-3*$^{L}$ is indistinguishable from *rec-3*$^{+}$ in its level of control. This proves that there are minor differences between the *control* (*con*) genes, near to *am-1* and *his-2*, which recognize products of *rec-3* genes. Further, this is the first clear evidence, though indirect, that the binding sites for products of *rec* genes are situated in the chromosome regions where recombination is modulated.

## Introduction

Genes which control the local frequency of recombination in *Neurospora crassa* have been found at three distinct loci (Catcheside and Corcoran 1973; D. E. A. Catcheside 1974). The two wild strains, Emerson A and Emerson a, most commonly used as source material in our experiments, differ in respect to the genes at all three loci. In view of the ancestry of these two strains, summarized by Barratt (1962), it is useful to survey the distribution of these *recombination* genes in available wild strains and especially those in the pedigree of the most commonly used laboratory strains. These are Lindegren A, Lindegren a, Abbott 4A and Abbott 12a together with their derivatives Beadle and Tatum 1A, Beadle and Tatum 25a, Emerson A and Emerson a. Some further derivatives are included. The results throw doubt on the authenticity of some of the stocks available in collections.

This study has shown the source of the genes at each of the *rec-1*, *rec-2*, and *rec-3* loci. Additionally a third allele at the *rec-3* locus has been detected. It reduces allelic recombination at the *am-1* locus to a lesser degree than does *rec-3*$^{+}$.

## Materials and Methods

The sources of the wild strains whose *rec* constitutions were investigated are recorded in the tables where appropriate.

The methods used for crosses and for the analysis of progeny were based on those previously used (Catcheside 1966). A modification has been the omission of sorbose from the layer agar medium used in the assay of *amination-1*$^{+}$ prototrophs. Several stocks, especially Lindegren A and derivatives carrying its *rec-3* allele, appear to carry a factor leading to poor growth in the presence of sorbose. It may be situated near to the locus of *rec-3*. Reduction of sorbose concentration helps growth to a sufficient extent to allow reliable counts. The methods used for assay of the genes present in various

**Table 1. Summary of data for the determination of *rec* gene constitution in laboratory stocks**

Stock numbers in the collections kept by the Fungal Genetics Stock Center and D. G. Catcheside are in columns 2 and 3. Mean values of assays by methods described in the text are recorded in columns 4–7 together with (in brackets) the number of independent isolates on which each assay is based. The pedigree relationships of these stocks and their *rec* gene constitutions, inferred from these data, are shown in Fig. 1

| Stock | Stock numbers | | *rec-1* assay: | *rec-2* assays: | | *rec-3* assay: |
|---|---|---|---|---|---|---|
| | F.G.S.C. | D.G.C. | *his-1*$^+$ prototrophs per $10^5$ | *pyr-3/his-5* prototrophs (%) | *his-3*$^+$ prototrophs per $10^5$ | *am-1*$^+$ prototrophs per $10^5$ |
| Lindegren A | 853 | 7828 | (7) 2·06±0·40 | (2) 2·10±0·14 | | (16) 7·73±0·33 |
| | 853 | 14644 | (3) 1·05±0·22 | (3) 1·60±0·11 | | (4) 12·32±0·84 |
| Lindegren a | 541 | 7829 | (6) 1·53±0·18 | (2) 1·56±0·10 | | (7) 34·7 ±1·01 |
| Beadle and Tatum 1A | 354 | 7830 | (6) 1·64±0·20 | (2) 11·4 ±0·49 | | (8) 1·27±0·12 |
| Beadle and Tatum 25a | 353 | 7831 | (4) 1·31±0·21 | (2) 1·16±0·06 | | (6) 8·43±0·37 |
| Abbott 4A | | 832 | | | | (8) 0·48±0·07 |
| | 1757X | 14561 | (6) 28·34±0·94 | (3) 0·68±0·04 | | (3) 1·13±0·18 |
| | 1758 | 14032 | (6) 27·04±1·11 | (4) 0·55±0·02 | | (4) 1·68±0·15 |
| Abbott 12a | 1758X | 14562 | (3) 2·07±0·26 | (5) 1·26±0·08 | | (3) 27·4 ±1·7 |
| | 1757 | 14031 | (6) 1·47±0·18 | (4) 1·50±0·08 | | (4) 20·6 ±0·7 |
| Emerson 5256A | 424 | 14030 | (6) 1·31±0·24 | (2) 10·8 ±0·47 | | (2) 1·78±0·35 |
| 'Emerson A' | 691 | 1534 | (7) 29·8 ±1·23 | (63) 0·74±0·01 | (32) 4·48±0·15 | (10) 1·25±0·07 |
| Emerson 5297a | 352 | 14029 | (5) 1·18±0·23 | (2) 13·9 ±0·72 | | (2) 30·1 ±1·7 |
| 'Emerson a' | 692 | 1535 | (14) 1·60±0·16 | (116) 11·79±0·08 | (11) 92·8 ±2·3 | (2) 23·0 ±0·83 |
| St. Lawrence 74A | | 3474 | (2) 2·88 | | (5) 4·68±0·64 | (12) 1·0 ±0·08 |
| St. Lawrence 79a | 533 | 7832 | | (4) 1·24±0·15 | | (4) 6·29±0·58 |
| RL 3–8A | 2218 | 14033 | (7) 1·18±0·17 | (2) 1·62±0·11 | | (2) 6·03±0·55 |
| RL 21a | 2219 | 14034 | (7) 1·56±0·19 | (2) 2·00±0·14 | | (2) 8·89±0·61 |

stocks at the *rec-1*, *rec2-* and *rec-3* loci all involved the transfer from each stock of the regions in which the genes are located into appropriate tester stocks.

(i) Genes at the *rec-1* locus were isolated by crossing the wild strains to stocks of the constitution K83 *ad-7 asp*. K83 is a *his-1* (*histidine-1*) allele; the *rec-1* locus is between *ad-7* (*adenine-7*) and *asp* (*asparagine*). From the crosses, progeny of the constitution K83 *ad-7*$^+$ *asp*$^+$ were isolated. These would have the region, including the *rec-1* locus, derived from the particular wild strain. These progeny were crossed to K651 *rec-1* testers and the frequency of *his-1*$^+$ recombinants was determined by selective plating of the resultant ascospores.

(ii) Genes at the *rec-2* locus were isolated by crossing the wild strains to stocks of the constitutions *pyr-3 sp am-1*$^6$ or *his-5 sp am-1*$^6$ and isolating *pyr-3* (*pyrimidine-3*) or *his-5* progeny, respectively, that are *sp*$^+$ *am-1*$^+$, from these crosses. The locus of *rec-2* is between *sp* (*spray*) and *am-1* (*amination-1*), so the gene present in a particular wild strain is recovered with *pyr-3* or *his-5*. Tests were made by crossing these progeny to *his-5 rec-2* or *pyr-3 rec-2* testers. The frequency of recombination between *pyr-3* and *his-5* was measured in these tests.

In some cases, the gene present at the *rec-2* locus in a given wild strain was determined by crossing the wild strain to a *his-3 am-1* stock (usually K874 *cog*$^+$ *am-1*$^6$) and isolating *his-3* progeny. These were crossed to a suitable *his-3 rec-2* tester (usually K504 *cog rec-2*) and the frequency of *his-3*$^+$ prototrophs determined in the crosses.

(iii) Genes at the *rec-3* locus, situated in linkage group I between the *acr-3* and *arg-3* loci, were isolated by crossing to stocks, of the appropriate mating type, which were a *arg-3 am-1*$^6$, A *arg-3 am-1*$^6$, a *his-2 am-1*$^6$ or A *his-2 am-1*$^6$. From these crosses progeny of the constitution *arg-3*$^+$ *his-2*$^+$ *am-1*$^6$ and of the same mating type as the parental wild strain were isolated. These progeny were crossed to *am-1*$^6$ *rec-3* testers and the frequency of *am-1*$^+$ prototrophs determined in the crosses.

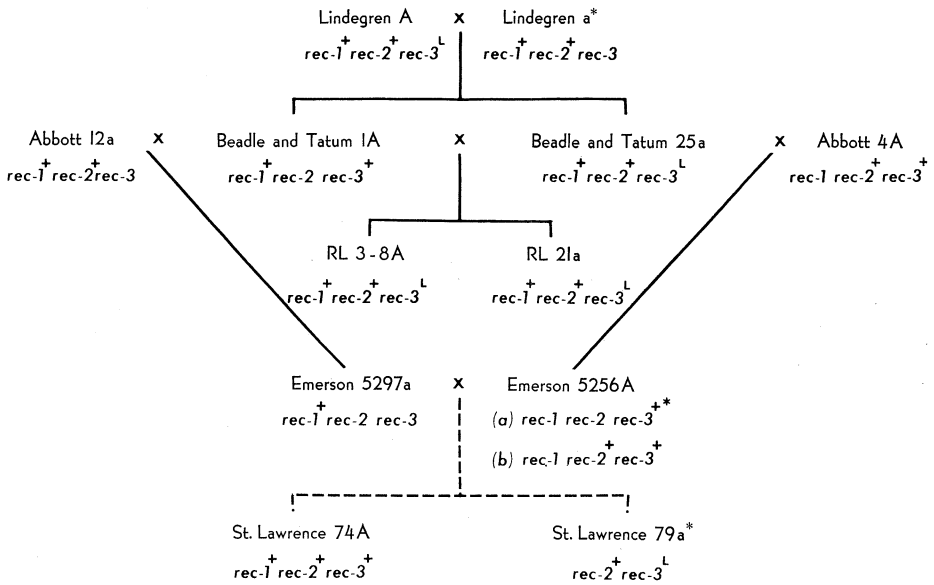



**Fig. 1.** Pedigree of commonly used laboratory stocks of *Neurospora crassa*. The constitutions in respect of genes at the *rec-1*, *rec-2* and *rec-3* loci are derived from data in Table 1. The conserved stocks marked with an asterisk have constitutions incompatible either with their reputed ancestry or their progeny.

## Results

### (*a*) *The* rec *Genes Present in Wild and Laboratory Stocks*

The data assembled for the various wild stocks and the laboratory stocks prepared by intercrossing these are summarized in Table 1. The pedigree relationships between

the various wild stocks and their derivatives, as recorded by Barratt (1962), are set out in Fig. 1 in which the *rec* gene constitutions derived from the data in Table 1 are incorporated.

At the present time, three *rec* loci are known (Catcheside and Corcoran 1973). Until now, two alleles were known at each locus. The standard representatives are those in 'Emerson A' (DGC 1534) and 'Emerson a' (DGC 1535); Emerson A is *rec-1 rec-2*$^+$ *rec-3*$^+$ while Emerson a is *rec-1*$^+$ *rec-2 rec-3*. The data in Table 1 are consistent with there being only the two previously known alleles at each of the *rec-1* and *rec-2* loci. Nevertheless, the variation in the low frequency values of recombination between *pyr-3* and *his-5* could be due to the existence of more than one *rec-2* allele capable of repressing recombination. Equally well it could be due to other genes of minor effect modulating the expression of the *rec-2*$^+$ gene. The latter possibility is consistent with the observation that variations to the extent recorded are found in tests of different isolates containing the same *rec* allele (Catcheside 1971).

The situation at the *rec-3* locus is different, in that there is *prima facie* evidence of a third allele, *rec-3*$^L$, present in Lindegren A and some of its derivatives and having an effect intermediate between that of *rec-3* and *rec-3*$^+$. Roughly, the relative effects are that, in the test of recombination between *am-1*$^2$ and *am-1*$^6$, the frequency of *am-1*$^+$ prototrophs per $10^5$ progeny is about 25 in *rec-3* $\times$ *rec-3*, about 1 in *rec-3*$^+$ $\times$ *rec-3*, and about 8 in *rec-3*$^L$ $\times$ *rec-3*. The evidence that the intermediate value is indeed due to a third allele at the *rec-3* locus, rather than to a gene at a different locus modifying the effect of *rec-3*$^+$ or *rec-3*, is presented in Results, section (*c*).

(*b*) *Pedigree of Wild and Laboratory Stocks*

There are four original wild stocks from which the principal laboratory stocks are descended. These are Lindegren A, Lindegren a, Abbott 12a, and Abbott 4A. It is evident that the stock preserved in the Fungal Genetics Stock Centre as 'Lindegren a' is not the original. It is *albino* and also has an unusual morphological character. Judging from the constitution of the two Beadle and Tatum stocks, 1A and 25a, derived from interbreeding Lindegren A and Lindegren a, the constitution of Lindegren a probably was *rec-1*$^+$ *rec-2 rec-3*$^+$. The stock analysed and presently conserved in stock collections differs from this inferred constitution in respect of two loci; it is *rec-1*$^+$ *rec-2*$^+$ *rec-3*.

Emerson 5297a has a constitution consistent with its ancestry, having *rec-2* from Beadle and Tatum 1A and *rec-3* from Abbott 12a. On the other hand, the FGSC stock of Emerson 5256A is not consistent with its ancestry, since it has *rec-2* instead of the expected *rec-2*$^+$. The records of the Fungal Genetics Stock Centre show that this stock (number 424) was founded from a "supposed original transfer from the University of Texas from [Professor] Wagner's laboratory", as Professor S. Emerson comments. It further appears that some re-selection may have occurred in Texas. The stock DGC 1534 is descended from a stock of Emerson 5256A sent to me in 1948 by Professor Beadle. However, it is uncertain whether DGC 1534 is a direct vegetative descendant or the product of an outcross to Emerson 5297a and repeated backcross to Emerson 5256A. The stock DGC 1534 has the right constitution to be a descendant of Beadle and Tatum 25a $\times$ Abbott 4A, while FGSC 424 has the wrong constitution.

St. Lawrence 74A has a constitution compatible with its ancestry, if it is conceded that the original Emerson 5256A was *rec-1 rec-2*$^+$ *rec-3*$^+$. However, the stock labelled

as St. Lawrence 79a is a rogue. It grows slowly, but is stimulated by thiamine. Crosses are slow in establishment and half the spores are defective, failing to pigment and ripen. Cultures from mature spores grow slowly and difficulties were encountered in obtaining progeny for the assay of the *rec* genes in FGSC 541.

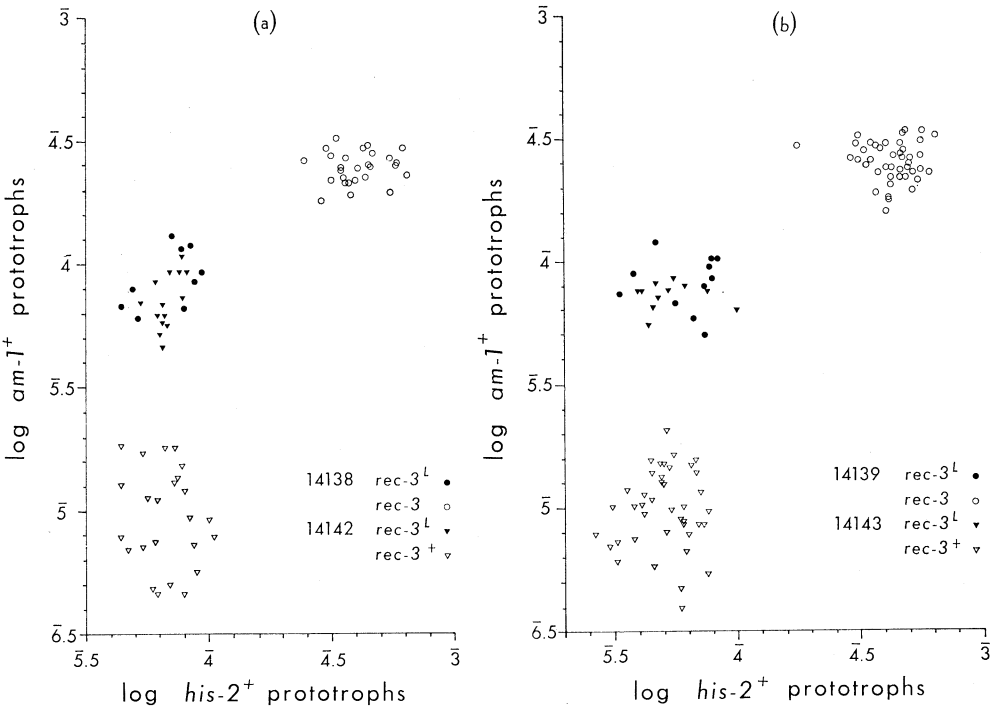



**Fig. 2.** (*a*) and (*b*) Plots of the logarithms of the frequencies of recombination at *am-1* locus and *his-2* locus in progeny of the crosses *rec-3*$^{L}$ × *rec-3*$^{+}$ and *rec-3*$^{L}$ × *rec-3* at each locus for each individual analysed. Symbols are as indicated in the figures.

### (*c*) *Genetics of* rec-3 *Lindegren*

It is evident that Lindegren A has a genetic factor which results in different frequencies of allelic recombination at the *am-1* locus from those in *rec-3* × *rec-3* or *rec-3*$^{+}$ × *rec-3* crosses. Moreover, this factor must be close to the *rec-3* locus since the effect is regularly isolated with the region containing the *rec-3* locus. It has seemed simplest to test the assumption that the genetic factor in Lindegren A is due to an allele, *rec-3*$^{L}$, at the *rec-3* locus. At the same time, its effect at the *his-2* locus was also investigated. To this end stocks (14069 and 14070) of the constitution A *rec-3*$^{L}$ K584 *am-1*$^{6}$ *cot-1* were bred, K584 being a *his-2* allele. Each of these was crossed to two other stocks, viz. 12572 which is a *acr-3*$^{r}$ *rec-3 arg-3 cot-1 rec-2* and 12573 which is a *acr-3*$^{r}$ *rec-3*$^{+}$ *arg-3 cot-1 rec-2*. These two stocks were bred respectively from 9980 and 9981 (used by Catcheside and Austin 1971) by crossing each of these to 3820 (A *cot-1 rec-2 am-1*$^{+}$) and replacing the *rec-2*$^{+}$ and *am-1*$^{2}$ genes in them by selecting progeny which did not require alanine for growth.

In the four crosses (14138, 14139, 14142, and 14143) generated as described, the frequency of recombination between *acr-3*$^{r}$ and *arg-3* was measured (Table 2) by

Fig. 3

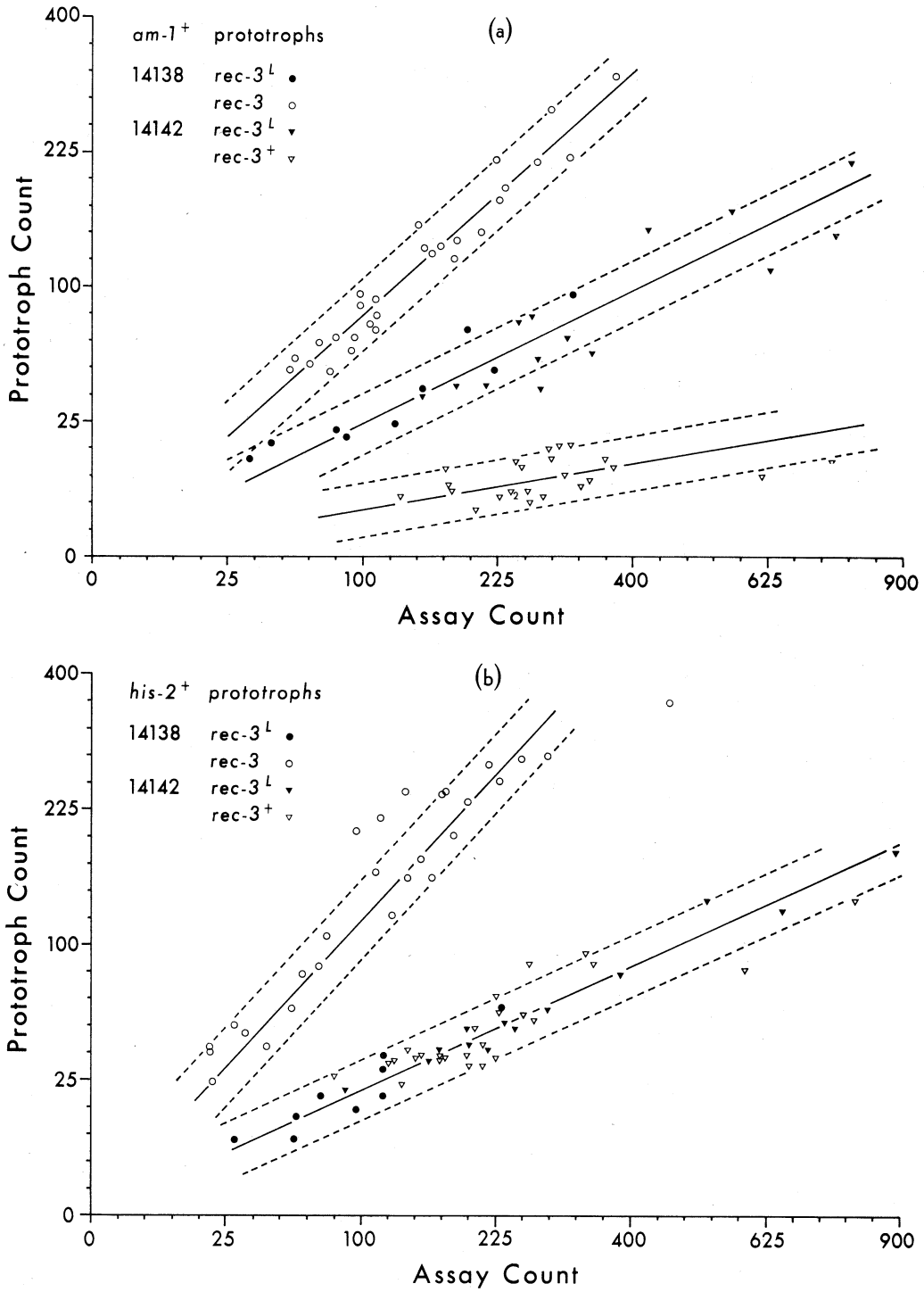
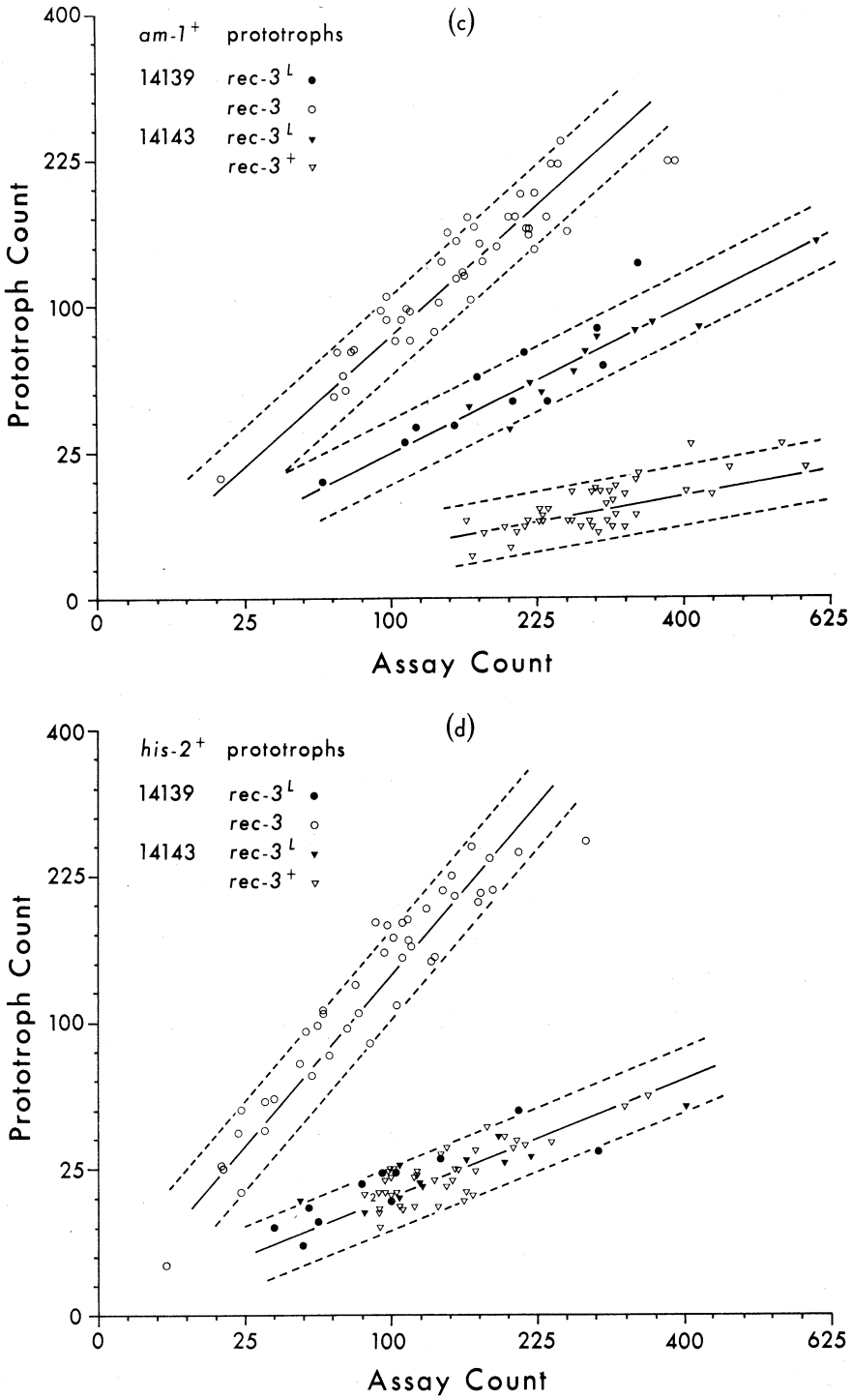



**Fig. 3.** (*a*)–(*d*) Plots of the square roots of the prototroph count and the assay count at a constant dilution for progeny of the crosses *rec-3*$^{L}$ × *rec-3*$^{+}$ and *rec-3*$^{L}$ × *rec-3* for each individual for each

**Fig. 3**




locus separately, the solid line being the mean slope and the dashed lines the 95% confidence limits. Symbols are as indicated in the figures.

selecting recombinants resistant to acriflavine and independent of arginine for growth. Most of the recombinants required histidine and a half of them required alanine for growth. Each of a large sample of these a *acr-3*$^r$ K584 *am-1*$^6$ *cot-1* progeny was crossed to (1) 3675 : A *am-1*$^2$ *cot-1 rec-3* and (2) 7575 : A K612 *cot-1 rec-3*, K612 being another *his-2* allele. These crosses were analysed respectively for the frequency of prototrophs formed at the *am-1* and *his-2* loci. The results are summarized in Figs 2 and 3 and in Tables 2 and 3.

**Table 2. Percentage recombination in the *acr-3 arg-3* segment and classification for allelic constitution at the *rec-3* locus of selected recombinants in the *acr-3 arg-3* interval, having the constitution a *acr-3*$^r$ *arg-3*$^+$ *his-2* K584 *am-1*$^2$ obtained from various crosses**

The numbers of progeny arising from recombination in the *acr-3 rec-3* segment are given in bold type. Data for crosses 10154 and 10155 have been published previously

| Cross No. | Recombination (%) in *acr-3 arg-3* segment | *rec-3* alleles in progeny *rec-3* | *rec-3*$^+$ | *rec-3*$^L$ |
|---|---|---|---|---|
| 10154 | 2·20±0·11 | 43 | **15** | |
| 10155 | 2·49±0·13 | **30** | 71 | |
| 14138 | 5·09±0·42 | 28 | | **9** |
| 14139 | 7·71±0·72 | 44 | | **11** |
| 14142 | 4·74±0·32 | | 24 | **14** |
| 14143 | 6·97±0·42 | | 41 | **11** |

Parentage of crosses

| | | | |
|---|---|---|---|
| 10154 | A *acr-3*$^s$ *rec-3*$^+$ + *his-2*; + (7576) | × | a *acr-3*$^r$ *rec-3 arg-3* +; *am-1*$^2$ (9980) |
| 10155 | A *acr-3*$^s$ *rec-3* + *his-2*; + (7575) | × | a *acr-3*$^r$ *rec-3 arg-3* +; *am-1*$^2$ (9981) |
| 14138 | A *acr-3*$^s$ *rec-3*$^L$ + *his-2*; *am-1*$^6$ (14069) | × | a *acr-3*$^r$ *rec-3 arg-3* +; + (12572) |
| 14139 | A *acr-3*$^s$ *rec-3*$^L$ + *his-2*; *am-1*$^6$ (14070) | × | a *acr-3*$^r$ *rec-3 arg-3* +; + (12572) |
| 14142 | A *acr-3*$^s$ *rec-3*$^L$ + *his-2*; *am-1*$^6$ (14069) | × | a *acr-3*$^r$ *rec-3*$^+$ *arg-3* +; + (12573) |
| 14143 | A *acr-3*$^s$ *rec-3*$^L$ + *his-2*; *am-1*$^6$ (14070) | × | a *acr-3*$^r$ *rec-3*$^+$ *arg-3* +; + (12573) |

**Table 3. Mean frequencies of prototrophs per $10^5$ progeny in the test crosses of the recombinants summarized in Table 2, together with data from previous experiments**

The *am-1*$^+$ prototrophs are recombinants between *am-1*$^2$ and *am-1*$^6$; the *his-2*$^+$ prototrophs are recombinants between K584 and K612. Data for crosses 10154 and 10155 have been published previously

| Cross No. | *am-1*$^+$ progeny *rec-3* | *rec-3*$^L$ | *rec-3*$^+$ | *his-2*$^+$ progeny *rec-3* | *rec-3*$^L$ | *rec-3*$^+$ |
|---|---|---|---|---|---|---|
| 10154 | 33·0±0·4 | — | 0·8±0·1 | 51·0±2·3 | — | 5·9±0·3 |
| 10155 | 30·7±0·6 | — | 1·2±0·1 | 41·3±0·8 | — | 6·2±0·1 |
| 14138 | 25·0±0·6 | 8·6±0·5 | — | 38·2±0·9 | 7·2±0·6 | — |
| 14139 | 24·9±0·4 | 8·5±0·4 | — | 42·7±0·9 | 5·8±0·4 | — |
| 14142 | — | 7·3±0·2 | 0·9±0·1 | — | 6·7±0·2 | 6·5±0·2 |
| 14143 | — | 7·3±0·3 | 1·1±0·1 | — | 5·0±0·3 | 5·0±0·2 |

Tables 2 and 3 also contain for comparison a summary of the results of two analyses previously published (Catcheside and Austin 1971; Catcheside and Corcoran 1973). It is evident that recombination between *acr-3* and *arg-3* in these two crosses was only a half or a third of that in the more recent crosses (14138, 14139, 14142, and 14143).

It is possible that this effect is due to *rec-2*$^+$ which is known to halve recombination in the *arg-3 sn* segment (Catcheside and Corcoran 1973). All of the parents of the four recent crosses are *rec-2*, whereas at least one of the parents of each of the two previous crosses was *rec-2*$^+$. The effect appears to be uniform over the *acr-3 arg-3* segment since the relative position of the *rec-3* locus is the same in all crosses; the heterogeneity $\chi^2$ is 4·687 for five degrees of freedom. The locus of *rec-3* is approximately a quarter (more exactly 0·264) of the genetic distance from *acr-3* to *arg-3*.

The test crosses by which the selected progeny were classified showed in several sets an exceptional degree of divergence from the average. The method used in these assays was to suspend the spores from one crossing tube in 20 ml of layer agar and to dilute a sample of this suspension 1600-fold. Four 3-ml samples of the undiluted suspension were plated on selective medium (A plates) and two 3-ml samples of the diluted suspension were plated on unselective medium (C plates). The number of colonies growing on the A plates is an estimate of the number of prototrophs present in a population whose size can be calculated from the number of colonies on the C plates. The ratio of the A count to the C count should be constant for a given set of tests, e.g. for *rec-3*$^L$ × *rec-3* crosses. Hence the homogeneity can be tested by calculating the heterogeneity $\chi^2$ values and the data can also be displayed visually in square root charts (Figs 3*a*–3*d*). These procedures showed the presence of some heterogeneity in the primary data. One genetic cause is the occasional occurrence of a reversion in one of the stocks, either tester or tested. If this occurs early in the growth of a culture, spuriously high values of prototroph frequency are shown. A second cause could be the segregation of genetic factors which slightly modify the recombination frequency. These appear as small, but consistent, differences between crosses as manifested by the within-column variation in Table 3 and the variation within sets in Figs 2 and 3. The effects are probably due to genetic causes of minor effect arising from the four different parents of the crosses. Nevertheless, these variations do not obscure the classification into *rec-3*, *rec-3*$^L$ and *rec-3*$^+$ categories when the effects on the *am-1* locus are used.

The primary data were analysed to identify the test crosses which agreed least well with the rest of the data. Every cross was repeated if it was on or outside the 95% confidence limits of the class to which it was presumed to belong. In all there were 85 which had to be re-examined, 36 for *am-1*$^+$ and 49 for *his-2*$^+$. Not only were the exceptions more frequent for *his-2*$^+$, but the departures in many cases were very large, due to the rather more frequent reversion of the *his-2* genes. In the total of 364 tests, about 18 cases outside the 95% confidence limits would have been expected.

Further studies of all doubtful assays yielding, with the previously acceptable data, the results summarized in Figs 2 and 3, have shown a number of progeny whose values for *am-1*$^+$ or *his-2*$^+$ prototrophs fall outside, sometimes well outside, the 95% confidence limits of one of the classes. However, in no instance did the values for the *am-1* and the *his-2* assays of any individual both fall outside their expected limits. Hence there is no clear evidence of a *rec-3* allele different from the known three, as could be expected to arise by rare recombination between allelic genes.

In the case of the frequency of *am-1*$^+$ prototrophs the data for progeny of *rec-3*$^L$ constitution were combined (14138 + 14142 and 14139 + 14143, respectively) in computing means and confidence limits (Figs 2 and 3). In the case of the frequency of *his-2*$^+$ protrotrophs, the data for progeny of *rec-3*$^L$ and *rec-3*$^+$ constitution were

similarly combined in computing means and confidence limits. The results show quite clearly that *rec-3*$^{L}$ and *rec-3*$^{+}$ do not have differential effects at the *his-2* locus.

## Discussion

Four independent wild strains of *N. crassa*, namely Lindegren A, Lindegren a, Abbott 4A, and Abbott 12a, between them are the sources of the pool of *recombination* genes present in commonly used laboratory stocks. Polymorphism is evident in respect to genes at three different loci which control levels of recombination in a number of different regions scattered in three different chromosomes. Two different alleles are present at each of the loci *rec-1* and *rec-2*, while there are three alleles at the *rec-3* locus. These conclusions are valid although the Lindegren a available in stock collections has a constitution inconsistent with those of progeny from a cross of true Lindegren a to Lindegren A. Instead of being *rec-1*$^{+}$ *rec-2*$^{+}$ *rec-3* it was probably *rec-1*$^{+}$ *rec-2 rec-3*$^{+}$.

The gene not previously described is *rec-3*$^{L}$, present in Lindegren A and many of its derivatives and characterized by its level of control of allelic recombination at the *amination-1* locus. Prototrophic recombinants occur in the relative proportions of 25 in *rec-3* × *rec-3*, 8 in *rec-3* × *rec-3*$^{L}$ or *rec-3*$^{L}$ × *rec-3*$^{L}$ and 1 in *rec-3* × *rec-3*$^{+}$ or *rec-3*$^{L}$ × *rec-3*$^{+}$ or *rec-3*$^{+}$ × *rec-3*$^{+}$. The *rec-3*$^{L}$ gene behaves as a dominant towards *rec-3* and as a recessive towards *rec-3*$^{+}$. It is perhaps surprising that *rec-3*$^{L}$ appears to be completely recessive to *rec-3*$^{+}$ since both produce products with an affinity, albeit different, for a target locus near to *am-1*. It may be that the marked difference in affinity is sufficient to account for the failure to differentiate between recombination at the *am-1* locus in *rec-3*$^{L}$ × *rec-3*$^{+}$ and *rec-3*$^{+}$ × *rec-3*$^{+}$ crosses. However, it appears that in every case one dose of a dominant *rec* gene is as effective as two. In contrast to the ability to distinguish between *rec-3*$^{L}$ and *rec-3*$^{+}$ in their control at the *am-1* locus, they show no differential effect on allelic recombination at the *his-2* locus nor on non-allelic recombination between the *his-2* and *sn* loci, as unpublished data show.

The evidence that *rec-3*, *rec-3*$^{L}$ and *rec-3*$^{+}$ are allelic is the apparent absence of recombination between any pair of them. Presumably, they are different from one another at one or more sites in the genes. At the least (Table 4i) it may be assumed that *rec-3* and *rec-3*$^{+}$ differ at only one site and that *rec-3*$^{L}$ differs from both at the same site. In this case no recombination can be expected from any pairwise cross between *rec-3*, *rec-3*$^{L}$ and *rec-3*$^{+}$. On the other hand, if *rec-3*$^{L}$ differs from either (Table 4ii, 4iii) or both (Table 4iv) of *rec-3* and *rec-3*$^{+}$ at two sites, possibilities arise of recombination of these differences. The phenotypes of some potential recombinants are predictable, but those of other recombinants are not. The recombinants of predictable phenotype arise when *rec-3*$^{L}$ differs from either of *rec-3* and *rec-3*$^{+}$ at one site and from the other at two sites. In this case *rec-3*$^{L}$ × *rec-3* (Table 4ii) could yield *rec-3*$^{+}$ and a new allele or *rec-3*$^{L}$ × *rec-3*$^{+}$ (Table 4iii) could yield *rec-3* and a new allele. All that can be said is that none of 92 progeny comprising a selected set from *rec-3*$^{L}$ × *rec-3* has been *rec-3*$^{+}$ and none of 90 progeny comprising a selected set from *rec-3*$^{L}$ × *rec-3*$^{+}$ has been *rec-3*. The selected sets are recombinants in a short segment within which the locus of *rec-3* lies (data in Table 2). Hence with 95% probability the frequency of formation of *rec-3*$^{+}$ by *rec-3*$^{L}$ × *rec-3* is less than 0·2%, while the frequency of formation of *rec-3* by *rec-3*$^{L}$ × *rec-3*$^{+}$ is less than 0·19%, if either of these events is possible. The differences between *rec-3*$^{L}$ and either of *rec-3* and *rec-3*$^{+}$

have not been recombined in substantial populations. The equivalent unselected populations in which recombinants have been sought are calculated to be 1440 for *rec-3* and 1548 for *rec-3*$^+$. There is no definite evidence of any distinct and new type of recombinant allele. In the logarithmic plots, no individual falls outside one of the three populations identifiable as *rec-3*$^+$, *rec-3*$^L$ or *rec-3*. The apparent exception in 14139 is due to the very poor fertility of the test crosses, especially with the *his-2* tester. Whether any of the individual progeny which depart significantly from the means in the square root plots can be regarded as due to changes in the *rec-3* genes is doubtful. They would require testing along the lines and to the detail pursued in examining *rec-3*$^L$.

**Table 4. Possible structural differences between *rec-3*$^L$, *rec-3* and *rec-3*$^+$ and the rare recombinants possible from hybrids**

Rare recombinants shown in bold type

| | | *rec-3*$^+$ × *rec-3*$^L$ | *rec-3* × *rec-3*$^L$ |
|---|---|---|---|
| (i) | *rec-3*$^L$ differs at one site from both | | |
| | *rec-3*$^+$ a | | |
| | *rec-3* b | a | a |
| | *rec-3*$^L$ c | c | b |
| (ii) | *rec-3*$^L$ differs at two sites from *rec-3* | | |
| | *rec-3*$^+$ y a | ay | by |
| | *rec-3* y b | ax | ax |
| | *rec-3*$^L$ x a | | **ay** = *rec-3*$^+$<br>**bx** = new 3 |
| (iii) | *rec-3*$^L$ differs at two sites from *rec-3*$^+$ | | |
| | *rec-3*$^+$ y a | ay | |
| | *rec-3* y b | bx | by |
| | *rec-3*$^L$ x b | **by** = *rec-3*<br>**ax** = new 1 | bx |
| (iv) | *rec-3*$^L$ differs at two sites from both | | |
| | *rec-3*$^+$ y a | ay | by |
| | *rec-3* y b | cx | cx |
| | *rec-3*$^L$ x c | **ax** = new 1<br>**cy** = new 2 | **bx** = new 3<br>**cy** = new 2 |

In previous work (Catcheside and Austin 1971; Catcheside and Corcoran 1973) it was concluded that genes at the *rec-3* locus also control allelic recombination at the *his-2* locus and non-allelic recombination between the *sn* and *his-2* loci. The alternative was that recombination in the *his-2* region is under the control of genes at a *rec-x* locus distinct from *rec-3* but very close to it, not more than about 0·05 centimorgans away with 95% probability, and with all stocks either *rec-3 rec-x* or *rec-3*$^+$ *rec-x*$^+$. No recombinants between the *rec-3* and *rec-x* loci were observed in the equivalent of 6692 unselected progeny. Allelic recombination at the *his-2* locus is the same in *rec-3*$^L$ ×

*rec-3* crosses as in *rec-3*$^+$ × *rec-3* crosses. It could be argued that the constitution of Lindegren A is *rec-3*$^L$ *rec-x*$^+$ and therefore in crosses to stocks hypothetically *rec-3 rec-x* it should be possible to obtain readily recognizable recombinants. These should be (1) *rec-3 rec-x*$^+$ characterized by 25 *am-1*$^+$ and 7 *his-2*$^+$ prototrophs per $10^5$ in standard test crosses to *rec-3 rec-x* and (2) *rec-3*$^L$ *rec-x* characterized by 8 *am-1*$^+$ and 42 *his-2* prototrophs per $10^5$ ascospores in the standard test crosses to *rec-3 rec-x*. No recombinants of these kinds have been found among the 92 progeny from *rec-3*$^L$ × *rec-3* crosses, so on these data the hypothetical *rec-x* locus is less than 0·2 centimorgans from the *rec-3* locus with 95% probability.

The simplest assumption is that there is one locus (*rec-3*) at which various controlling genes, such as *rec-3*, *rec-3*$^L$ and *rec-3*$^+$, have differential effects upon recombination in the neighbourhoods of the *am-1* and *his-2* loci. Their localized effect requires the presence of recognition sites (*con-3* genes) in the neighbourhood of *am-1* and of *his-2* (Catcheside 1966; Angel *et al.* 1970). These need not be exactly alike, so *con-3-am-1* and *con-3-his-2* could have different relative affinities for the products of *rec-3*, *rec-3*$^L$ and *rec-3*$^+$. Identical affinity for the products of *rec-3*$^L$ and *rec-3*$^+$ accounts for the absence of any difference shown by allelic recombination at the *his-2* locus caused by the substitution of *rec-3*$^+$ for *rec-3*$^L$. In contrast differential affinity accounts for the large effect shown at the *am-1* locus. So far variants of the *con* loci in the neighbourhoods of *am-1*, *his-1*, *his-2* or *his-3* have not been observed.

In discussing the regulation of recombination in the neighbourhood of the *his-3* locus (Angel *et al.* 1970), it was concluded that the available evidence was more readily compatible with there being two distinct types of *recognition* loci, one (*con*) for the product of the *rec* genes and the other (*cog*) for a presumed recombinase, probably an endonuclease responsible for a nick in the *cog* gene. A variant (*cog*$^+$) at the *cog* locus, capable of more frequent recognition and nicking by recombinase, behaves as a dominant to a lesser variant and the dominant allele raises recombination locally. The *recognition* locus (*con*) for the product of *rec* genes could either be an operator near the recombinase gene (*nuc*, for nuclease) or near the region subject to recombination control. In the latter case it would control access of recombinase to the *cog* locus or unravelling of the nicked DNA as a prelude to recombination. If a *con* gene were an operator of a recombinase, so that the amount of this enzyme was controlled by the interaction of *con* and the products of *rec-3*, *rec-3*$^L$ and *rec-3*$^+$, it would be expected that differential effects on different target loci would be proportionate. For example, taking the relative yields of prototrophs at the *am-1* locus as being 1, 8 and 25 for *rec-3*$^+$, *rec-3*$^L$ and *rec-3* respectively, the expectation at the *his-2* locus would be 6, 17 and 40 rather than the 6, 6 and 40 observed. The conclusion is that the targets of the products of *rec* genes are not operators of *recombinase* (*nuc*) genes, but rather are control (*con*) genes in the target regions. There would be a number of species of *con* gene, each species corresponding to a *rec* locus; thus, *con-1* for *rec-1* and *con-3* for *rec-3*. There would be a number of loci, distributed through the chromosomes, each with a variety of a given species of *con*. However, the different varieties would not all be exactly alike. They would differ in their relative affinities for the products of different allelic *rec* genes. For example, the product of *rec-3* has a relatively low affinity for *con-3-am-1* and for *con-3-his-2*, the product of *rec-3*$^L$ has a higher affinity for both *con* genes and the product of *rec-3*$^+$ has a very high affinity for *con-3-am-1*. The products of *rec-3*$^L$ and *rec-3*$^+$, on the other hand, do not have different affinities for *con-3-his-2*.

## References

Angel, T., Austin, B., and Catcheside, D. G. (1970). Regulation of recombination at the *his-3* locus in *Neurospora crassa*. *Aust. J. Biol. Sci.* **23**, 1229–40.

Barratt, R. W. (1962). Origin of important wild type stocks of *N. crassa*. *Neurospora Newsletter* **2**, 24–5.

Catcheside, D. E. A. (1974). A second locus subject to recombination control by the *rec-1*$^+$ gene in *Neurospora crassa*. *Aust. J. Biol. Sci.* **27**, 561–73.

Catcheside, D. G. (1966). A second gene controlling allelic recombination in *Neurospora crassa*. *Aust. J. Biol. Sci.* **19**, 1039–43.

Catcheside, D. G. (1971). Regulation of crossing over in *Neurospora crassa*. In 'Genetics Lectures'. (Ed. R. Bogart.) Vol. 2. pp. 7–18. (Oregon State University Press.)

Catcheside, D. G., and Austin, B. (1971). Common regulation of recombination at the *amination-1* and *histidine-2* loci in *Neurospora crassa*. *Aust. J. Biol. Sci.* **24**, 107–15.

Catcheside, D. G. and Corcoran, D. (1973). Control of non-allelic recombination in *Neurospora crassa*. *Aust. J. Biol. Sci.* **26**, 1337–53.

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