Heterogeneity in recombination frequencies in *Neurospora crassa*

By L. C. FROST

Genetics Laboratory, Department of Botany,
University of Bristol

1. INTRODUCTION

In the past diverse wild-type stocks of *Neurospora crassa* have been used by workers. Lindegren (1932 *a*) used only two wild strains of opposite mating-type, but Beadle & Tatum (1945) also introduced the Abbott and Chilton stocks in order to maintain vigour on intercrossing (Beadle—personal communication). Emerson & Cushing (1946) and St Lawrence (De Serres, 1958 *b*) produced new 'wild-types' by hybridizing Abbott and Lindegren strains. The origin of the wild-type strains and the wild-type ancestry of the originally isolated biochemical mutant strains is known (Table 1). However, as few records have been kept of re-isolations, the laboratory stocks of today are of unknown but highly mixed wild-type ancestries.

In their linkage studies Houlahan, Beadle & Calhoun (1949) pooled data from several crosses involving a single locus. When these data were tested, marked heterogeneity was present in the centromere distance of the majority of the loci (Frost, 1955 *b*). The first report of substantial strain differences in the centromere distance of a locus was by Teas (1947). Barratt, Newmeyer, Perkins & Garnjobst (1954), Holloway (1954) and Stadler (1956 *a*) reported heterogeneity in centromere distances based on ordered tetrads. In random spore analyses, discrepancies or heterogeneity in recombination frequencies between crosses of the same linked markers were reported by Mitchell & Mitchell (1954), Frost (1955 *b*), Rifaat (1956, 1958), Mitchell (1958), De Serres (1958 *a*) and Perkins (1959).

Barratt (1954) summarized the data on heterogeneity in centromere distance of loci including instances from almost every linkage group and pointed out that this was associated with the presence in the crosses of an Abbott stock as opposed to a Lindegren stock. Significant reduction of centromere distance over that from crosses of two Lindegren stocks occurred in crosses of Abbott 4 and Lindegren derivation for *mt*, *al-1*(4637T), *me-3*(36104), *nt*(39401) and *chol-1*(37903) but not for *inos*(37401), and in crosses of Abbott 12 and Lindegren derivation for *me-5*(9666) but not for *mt*, *arg-6*(29997) and *me-5*(36105). Significant increases in centromere distance over the Lindegren value occurred in crosses of Abbott 12 and Lindegren derivation for *pab-1*(1633) and for *mt* when either Abbott 4 or 12 was crossed with a mutant of Abbott and Lindegren derivation. Frost (1955 *b*) analysed in detail the data, including those kindly supplied by Dr R. W. Barratt, on heterogeneity in centromere distances and investigated further into the problem. Since this work

was not published and further experimental work has been carried out, the entire work is presented here. Heterogeneity in recombination frequencies has caused confusion in the study of the formal genetics of *Neurospora*. It is hoped that this report will be of value in clarifying the situation.

2. METHODS AND MATERIALS

Nomenclature

The system of designating strains will be that of Barratt *et al.* (1954), except that mating-type will be designated *mt* instead of *sex*, and strain 34508, *aur* instead of *al-1*.

Strains

The following strains were used, the origin being indicated by name after each strain.

Wild-types: Lindegren 1*A*, Lindegren 25*a*, Abbott 4*A*, Abbott 12*a*. (R. W. Barratt)

ad-3(35203), adenine. (R. W. Barratt)

al(G2), albino, believed allelic or pseudo-allelic with *aur*(34508). (B. M. Elliott)

al-2(15300), albino. (D. G. Catcheside)

arg-3(30300), arginine. (30300–68, D. G. Catcheside; R30300, A. M. Srb)

arg-6(29997), arginine. (D. G. Catcheside)

asco(37402), also known as *lys*. Requirement for lysine also ascospore abortion. (M. B. Mitchell)

nic-1(3416), nicotinic acid. (D. G. Catcheside)

Methods

The methods used were essentially those of Beadle & Tatum (1945). Crosses were made on the minimal reproductive medium devised by Westergaard & Mitchell (1947). Comparatively immature asci were dissected in an aqueous solution containing 1·5% w/v sodium hypochlorite and 1·5% v/v ethyl alcohol and were allowed to ripen for 10 days before heat treatment. To eliminate possibility of error, mating-type tests were carried out against standards of opposite mating-type. Inocula from each unknown culture was transferred into duplicate $3 \times \frac{3}{8}$ inch test-tubes; drops of conidial suspensions of standard *A* and *a* were then added to one and the other of the duplicates respectively.

3. SELECTION AND TREATMENT OF DATA

Only those data have been selected in which the wild-type ancestry of the strains crossed is known with certainty or was reported precisely. In addition the data must contain full details, for each individual cross, of the parentage of the cross together with the number of second-division segregations for the locus concerned, the total number of asci analysed and preferably also the total number of asci dissected since poor germination may give rise to biased results (Whitehouse, 1948).

Data fulfilling these essential requirements have been grouped according to wild-type ancestry of the crosses and each group tested statistically by means of the heterogeneity chi-squared test. If homogeneous, the totals of the first- and second-division segregations for a given locus of a certain ancestry type were compared by means of a two-by-two chi-squared test with the appropriate totals from crosses of pure Lindegren ancestry since the latter has been chosen as the standard wild-type ancestry against which to compare the behaviour of the other wild-type strains.

The origin or derivation of the wild-type strains and the wild-type ancestry of the mutant strains is given in Table 1. In some crosses of a given ancestry type,

Table 1. *Origin of the wild-type strains and wild ancestry of the mutant strains of* Neurospora crassa

Strain	Origin from	Ref.*
Lindegren *A*, Lindegren *a*	Material isolated by B. O. Dodge	1
1*A*, 10*a*, 19*a*, 25*a*	Lindegren *A* × Lindegren *a*	2
Abbott 4*A*, Abbott 12*a*	Material isolated by E. V. Abbott in Louisiana	2
Chilton *a*	Material isolated by St John P. Chilton in Louisiana	2
E5256*A*	Abbott 4*A* × 25*a*	3
E5297*a*	Abbott 12*a* × 1*A*	3
73*a*, 74*A*	E5256*A* × E5297*a* (P. St Lawrence)	4
308–952, 1274–26585	Lindegren *A* × Lindegren *a*	2
26586–35000	1*A* × 19*a*	2
35001–40100	1*A* × 25*a*	2
40101–44000, 60501–63000	Abbott 4*A* × Abbott 12*a*	2
44001–49000, 63001–*c.* 80000	Abbott 4*A* × 25*a*	2
49001–60500	Abbott 4*A* × Chilton *a*	2

* 1. Lindegren (1932*a*); 2. Beadle & Tatum (1945); 3. Emerson & Cushing (1946); 4. De Serres (1958*b*).

opposite mating-types of the same mutant strain were used. Beadle & Tatum (1945) stated that the mutant strains were each established from a single ascospore resulting from crossing irradiated material with a wild-type strain. Crosses were then made between these mutant strains and the wild-type from which they were derived. The author assumes therefore that it was from these latter crosses that both mating-types were obtained. In the case of mutant strains 308 to 40100 (Table 1) all such crosses would be of pure Lindegren ancestry. However, as regards mutant strains 40100 to 80000, it is not clear with which wild-type parent they were crossed in order to isolate both mating-types. The author has thus assumed that the ancestry of these strains is that given in Table 1. This may be an oversimplification of the wild-type ancestries involved but it does not fundamentally alter the situation. The data have been analysed where necessary to determine if opposite mating-types of the same mutant strain give significantly different results.

Besides data not fulfilling the essential requirements listed above, pooled data

from an unreported number of crosses and data from strains known to carry chromosome aberrations have been rejected.

4. HETEROGENEITY IN THE CENTROMERE DISTANCE OF THE MATING-TYPE LOCUS

The data given in Table 2 are arranged according to the wild-type ancestry of the strains crossed. The centromere distance of the Abbott 4 × Lindegren crosses shows a significant reduction from any of the other distances listed in the Table. The 'backcrosses' (Abbott 4 × Lindegren) × Lindegren show a significant decrease in centromere distance ($\chi^2 = 5\cdot210$, $n = 1$, $P = 0\cdot02$–$0\cdot05$) over the complementary backcrosses (Abbott 4 × Lindegren) × Abbott 4. The E5256 × Lindegren crosses are of (Abbott 4 × Lindegren) × Lindegren ancestry and show as expected no significant difference ($\chi^2 = 0\cdot403$, $n = 1$, $P = 0\cdot50$–$0\cdot70$) from the crosses of the latter ancestry type. The centromere distance of the (E5297 × Lindegren) selfed crosses is not significantly different ($\chi^2 = 0\cdot860$, $n = 1$, $P = 0\cdot30$–$0\cdot50$) from the E5297 × Lindegren value. However, Houlahan *et al.* (1949) listed a cross E5297*a* × 85902*A* which is probably of (Abbott 4 × Lindegren) × E5297 ancestry, giving a centromere distance for *mt* of $24\cdot3 \pm 4\cdot11$ (18/37) which is significantly increased ($\chi^2 = 5\cdot881$, $n = 1$, $P = 0\cdot01$–$0\cdot02$) over the E5297 × Lindegren value. It is the highest centromere distance of *mt* yet recorded in *N. crassa*.

It may be concluded from Table 2 that by grouping the crosses according to wild-type ancestry, heterogeneity in the centromere distance of *mt* is eliminated within any group.

Furthermore, the centromere distances of *mt* show similar values in crosses of Chilton compared with Lindegren ancestries but not in crosses of Abbott compared with Lindegren ancestries. Thus heterogeneity results from mixed wild-type ancestries involving an Abbott strain; significant increases over the Lindegren value being associated with an ancestry involving Abbott 12, while both significant increases and reductions result from the presence of Abbott 4. Therefore Abbott 4 is genetically different from Abbott 12, this being especially apparent in a direct cross to a Lindegren strain. As vegetative reproduction appears to predominate in nature, genetic differences between clones might well arise.

However, Lindegren *A* might differ genetically from Lindegren *a* rather than Abbott 4*A* from Abbott 12*a*. Against this supposition are the facts that the Lindegren strains used to obtain the above data have been inbred several times whereas the Abbott strains have not (Beadle & Tatum, 1945).

Furthermore, the author has intercrossed 1*A* with 25*a* and backcrossed some of the progeny to 1*A* for six successive backcrosses. Fertility is slightly reduced and some small variation in spore size results. When Abbott 12*a* was intercrossed with 1*A* and some of the progeny backcrossed to Abbott 12*a*, segregation occurred for ascospore abortion. Normal progeny were selected each time and backcrossed to Abbott 12*a* for five successive backcrosses. In each backcross generation, normal to aborted crosses occurred in approximately an equal ratio but there was much

Table 2. *The centromere distance of mt arranged according to wild-type ancestry of the crosses*

Data source[*]	Wild-type Ancestry[†]	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$P(\%)$	Centromere distance	$2 \times 2\chi^2$ with $(L \times L)$	$P(\%)$
1, 2, 3, 4, 5	$L \times L$	39	108	827	34·981	‡	6·53 ± 0·59	—	—
2, 4, 6, 7	$A4 \times L$	13	13	282	10·085	50–70	2·31 ± 0·62	15·445	< 1
4	$(A4 \times L) \times L$	17	42	298	15·370	30–50	7·05 ± 1·01	0·203	50–70
4	$(A4 \times L) \times A4$	8	32	140	4·757	50–70	11·43 ± 1·77	9·283	< 1
4	$E5256 \times L$	3	11	64	2·704	20–30	8·59 − 2·36	0·875	30–50
2, 4, 6, 8	$A12 \times L$	9	22	193	9·598	20–30	5·70 ± 1·14	0·388	50–70
3, 4	$E5297 \times L$	5	37	134	2·173	70–80	13·81 ± 1·93	19·062	< 1
3	$(E5297 \times L) \oplus$	10	55	169	11·133	20–30	16·27 ± 1·80	38·925	< 1
4	$(A4 \times L) \times A12$	4	20	77	0·623	80–90	12·99 ± 2·50	9·666	< 1
4	$C \times L$	2	6	50	3·834	5–10	6·00 ± 2·30	0·047	80–90
4	$(A4 \times L) \times C$	3	7	41	2·361	30–50	8·54 ± 2·94	0·548	30–50
4	$(A4 \times C) \times L$	4	7	86	4·879	10–20	4·07 ± 1·47	1·713	10–20

* 1. Lindegren (1932b, 1936); 2. Buss (1944); 3. Holloway (1953); 4. Houlahan *et al.* (1949); 5. Regnery (1947); 6. Srb (1946); 7. Doermann (1946); 8. Frost (1955b).

† A = Abbott; C = Chilton; E = Emerson; L = Lindegren; ⊕ = selfed.

‡ Not significant.

variation in spore size from minute colourless to giant black spores. When Abbott $4A$ was similarly investigated (S. Lavigne, personal communication), sterility in the first and subsequent backcrosses was even more pronounced and the shape of the ascospores was different, being narrower and with pointed ends. Hence it appears that both Abbott strains carry a gene or genes resulting in ascospore abortion in certain genetic backgrounds and that Abbott 4 shows differences from Abbott 12 in this respect.

In addition, some of the progeny of $1A \times 25a$ and $(1A \times 25a) \times 1A$ were crossed to the same isolate of $asco(37402)$. The crosses were homogeneous for the centromere distance of $asco$ (Table 9). However, progeny of Abbott $12a \times 1A$ crossed to the same isolate of $asco$ as above gave centromere distances of $asco$ which were significantly different (Table 8).

Hence it can be concluded that the presence of Abbott ancestry in crosses is the cause of heterogeneity in centromere distances, that the Abbott strains differ genetically from one another, and that the Lindegren strains of opposite mating-type give homogeneous results.

Finally direct crosses of a specific Abbott with a Lindegren strain give as expected homogeneous results since each ordered tetrad from such a cross reflects directly the similar recombinational events occurring during meiosis in each diploid ascus fusion nucleus. However, in the progeny of such a cross might segregate the factor(s) controlling different centromere distances if these are, in fact, genetically determined. Thus heterogeneous centromere distances might arise among such progeny when these are backcrossed to either parent, e.g. (Abbott $4 \times$ Lindegren) $\times$ Abbott 4.

It would appear from the homogeneity of the above backcross data that this is not the case, but if the data are plotted graphically by the method of Mosteller & Turkey (1949) a greater spread is apparent in the cases of the 'backcrosses' compared with the 'direct crosses'. When the centromere distance of mt, plus or minus twice its standard error, is plotted for each group of wild-type ancestry (Table 2), the distances fall into three significantly different groups of values (Frost, 1955 b). This suggests that if centromere distance is genetically determined, it is controlled among the various wild strains by three genetic factors or groups of factors.

5. HETEROGENEITY IN CENTROMERE DISTANCES OF LOCI IN LINKAGE GROUPS I TO VII

(a) Chilton crosses

The limited data are given in Table 3. The behaviour of the loci listed follows that of mt with the corresponding wild-type ancestries. Furthermore, the presence of Chilton as opposed to Lindegren genetic material introduces no further heterogeneity, again suggesting that these two wild strains show similar patterns of recombinational events.

(b) Abbott 12 crosses

The data are given in Table 4. It can be seen that the behaviour of the loci involved in crosses of Abbott $12 \times$ Lindegren ancestry also follow that of mt with

Table 3. *Centromere distance of loci from crosses involving Chilton a*

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$P(\%)$	Centromere distance	2×2 χ^2	$P(\%)$
1	*thi*(9185)	III	L×L	1	7	16	—	—	21·88 ± 6·20	0·012	90–95
			L×C	1	5	12	—	—	20·83 ± 7·17		
1	*pdx-1*(37803)	IV	L×L	6	37	199	10·175	5–10	9·30 ± 1·33	0·500	30–50
			(A4×C)×L	2	7	29	2·461‡	10–20	12·07 ± 3·97		
1	*pyr-2*(38502)	IV	L×L	2	30	46	1·304	20–30	32·61 ± 3·51	3·188	5–10
			(A4×C)×L	1	3	9	—	—	16·67 ± 7·86		
1, 2	*me-3*(36104)	V	L×L	3	33	60	1·212	50–70	27·50 ± 3·21	0·152	50–70
			(A4×C)×L	1	12	20	—	—	30·00 ± 5·48		
1	*nt*(39401)	VII	L×L	1	11	32	—	—	17·19 ± 4·20	0·279	50–70
			(A4×C)×L	1	4	15	—	—	13·33 ± 5·71		

* 1. Houlahan *et al.* (1949); 2. Buss (1944).

† A = Abbott; C = Chilton; L = Lindegren.

‡ 2×2 χ^2 test modified for small samples.

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Table 4. *Centromere distance of loci from crosses involving Abbott 12*

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$\hat{P}(\%)$	Centromere distance	2×2 χ^2	$P(\%)$
1	arg-6(29997)	I	L × L	1	20	32	—	—	31·25 ± 4·28	0·111	70–80
			L × A12	1	58	98	—	—	29·59 ± 2·48		
2	thi(9185)	III	L × L	1	7	16	—	—	21·88 ± 6·20	0·385	70–80
			L × A12	2	32	61	0·077	70–80	26·23 ± 3·20		
3	me-5(9666)	IV	L × L	5	50	87	0·947	90–95	28·74 ± 2·65	3·299	5–10
			L × A12	1	7	20	—	—	17·50 ± 5·33		
2	pyr-1(H263)	IV	L × L	2	13	108	0·045	80–90	6·02 ± 1·57	0·209	50–70
			L × A12	1	2	23	—	—	4·35 ± 2·94		
2	pdx-1(37803)	IV	L × L	6	37	199	10·157	5–10	9·30 ± 1·33	0·069	70–80
			L × A12	1	4	19	—	—	10·53 ± 4·68		
2	pab-1(1633)	V	L × L	1	7	16	—	—	21·88 ± 6·20	5·510	1–2
			L × A12	1	27	35	—	—	38·57 ± 3·55		
3	me-3(36105)	V	L × L	5	57	99	13·817	<1	c.28·8	—	—
			L × A12	1	12	20	—	—	30·00 ± 5·48		

* 1. Srb (1946); 2. Houlahan *et al.* (1949); 3. Buss (1944).
† A = Abbott; L = Lindegren.

corresponding ancestry. The only exception is *pab-1*(1633). Of the four crosses listed by Houlahan *et al.* (1949), one involves a translocation and another is of mixed Lindegren and Abbott 4 ancestry and so both have been rejected. The remaining two crosses are heterogeneous, and when the four-fold χ^2 test modified for small samples is used then $\chi^2 = 4.110$, $n = 1$, $P = 0.02-0.05$. Thus *pab-1* shows a significantly increased centromere distance in the Abbott 12 × Lindegren cross. With *me-3*(36105) the heterogeneity is present in the crosses of supposed pure Lindegren ancestry.

(c) *Emerson 5297 crosses*

The data are given in Table 5. Here the behaviour of some of the loci does not follow that of *mt* which showed a significant increase in centromere distance over the pure Lindegren value in E5297 crosses. In Group III, *thi*(9185) shows a significantly reduced centromere distance. Although Abbott 12 is concerned in the ancestry of E5297, *thi* showed no reduction in centromere distance in a cross to Abbott 12 (Table 4). Again, unlike *mt*, *pyr-2*(38502) shows no increase in centromere distance over the pure Lindegren value. However, the data for *aur* and *inos* come from the same cross, and thus both loci, although in different linkage groups, show simultaneously a significantly increased centromere distance compared with the pure Lindegren value.

This different pattern of heterogeneity does not appear to be associated with distance of the locus from its centromere. Perhaps the E5297 strain carries some chromosomal aberration or possibly if centromere distance is genetically determined the factor(s) concerned may act with different intensities in the different linkage groups.

(d) *Abbott 4 crosses*

The data are given in Table 6. Similarly to the case of *mt*, two out of four loci show significant reductions in centromere distance in Abbott 4 × Lindegren crosses as opposed to crosses of pure Lindegren ancestry. In the case of *chol-1* and its allele the Lindegren crosses are heterogeneous but nevertheless the Abbott 4 × Lindegren cross gives a much lower centromere distance. In addition *pab-1* and *nt* behave like *mt* in (Abbott 4 × Lindegren) × Lindegren crosses. In the case of *nt*, two crosses are given by Houlahan *et al.* (1949) but they are heterogeneous. One gave only 65% germination and so may be biased and has been discounted.

However, a tendency emerges, with mixed Abbott 4 and Lindegren ancestries, for loci proximal to their centromere to show a different pattern of heterogeneity in centromere distance from loci distal from their centromere. In Abbott 4 × Lindegren crosses, proximal loci show a significant reduction whereas distal loci do not, while in (Abbott 4 × Lindegren) × Lindegren crosses distal loci tend to show an increase whereas proximal loci do not. The distance from the centromere at which these phenomena take place might vary in the different linkage groups.

The data for *me-3* do not support this hypothesis, but if the four-fold χ^2 test for small samples is used, then $\chi^2 = 3.676$, $n = 1$, $P = 0.05-0.10$. Thus a reduction in

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Table 5. *Centromere distance of loci from crosses involving E5297*

Locus	Link group	Wild-type ancestry*	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$P(\%)$	Centromere distance	2×2 χ^2	$P(\%)$
aur(34508)	I	L × L [(A4 × L) × L] × E5297	12 1	141 93	270 122	12·301 —	30–50 —	26·11 ± 1·52 ⎱ 38·12 ± 1·93 ⎰	20·129	< 1
thi(9185)	III	L × L L × E5297	1 1	7 28	16 132	— —	— —	21·88 ± 6·20 ⎱ 10·61 ± 1·79 ⎰	4·015	2–5
pyr-2(38502)	IV	L × L L × E5297	2 1	30 12	46 20	1·304 —	20–30 —	32·61 ± 3·51 ⎱ 30·00 ± 5·48 ⎰	0·164	50–70
inos(37401) (83201)	V	L × L [(A4 × L) × L] × E5297	7 1	66 80	133 122	8·472 —	20–30 —	24·81 ± 2·17 ⎱ 32·79 ± 2·15 ⎰	6·614	1–2

All data from Houlahan *et al.* (1949).

* A = Abbott; E = Emerson; L = Lindegren.

Table 6. *Centromere distance of loci from crosses involving Abbott 4*

Data* source	Locus	Link group	Wild-type ancestry†	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$P(\%)$	Centromere distance	2×2 χ^2	$P(\%)$
1, 2	al-1(4637T)	I	L × L	15	33	298	19·715	10–20	5·54 ± 0·91	8·009	< 1
			L × A4	3	1	85	0·964	50–70	0·59 ± 0·58		
1	chol-1(37903)(34542)	IV	L × L	5	74	138	11·116	2–5	c. 26·8	—	—
			L × A4	1	5	30	—	—	8·33 ± 3·40		
1	pyr-2(38502)	IV	L × L	2	30	46	1·304	20–30	32·61 ± 3·51	0·084	70–80
			(A4 × L) × L	1	7	10	—	—	35·00 ± 7·25		
3	me-3(36104)	V	L × L	3	33	60	1·212	50–60	27·50 ± 3·21	4·756	2–5
			L × A4	1	5	19	—	—	13·16 ± 5·05		
1	inos(37401)	V	L × L	7	66	133	8·472	20–30	24·81 ± 2·17	0·008	90–95
			L × A4	1	21	43	—	—	24·42 ± 3·81		
1	pab-1(1633)	V	L × L	1	7	16	—	—	21·88 ± 6·20	1·706	10–20
			(A4 × L) × L	1	7	10	—	—	35·00 ± 7·25		
1	nt(39401)	VII	L × L	1	11	32	—	—	17·19 ± 4·20	1·019	30–50
			L × A4	1	4	19	—	—	10·53 ± 4·68		
1	nt(39401)	VII	L × L	1	11	32	—	—	17·19 ± 4·20	3·153	5–10
			(A4 × L) × L	2	15	26	1·348	20–30	28·85 ± 4·84		

* 1. Houlahan *et al.* (1949); 2. Srb (1946); 3. Buss (1944).
† A = Abbott; L = Lindegren.

centromere distance for *me-3* is barely significant. On the other hand, Barratt (1954) reported that Teas (1947) had observed a significantly increased recombination frequency for *aur*(34508) and *al-2*(15300) in crosses with *thr*(44104). These crosses are of (Abbott 4 × Lindegren) × Lindegren ancestry and thus do not behave similarly to *mt* but *aur* and *al-2* are distal from their centromere.

The data (Table 7) for *ad-4*(44206) and *ad-2*(70004t), both in the right arm of linkage group III, also show this effect of distance. The centromere distances from

Table 7. *Centromere distance of* ad-2 *and* ad-4 *both in the right arm of linkage group III from crosses of complex wild ancestries involving Abbott 4*

Locus	Cross* ancestry	No. of crosses	2nd-div. asci	Total asci	Het. χ^2	$P(\%)$	Centromere distance
ad-2(70004)	1	3	19	49	0·564	70–80	$19·39 \pm 3·48$
	2	2	17	32	0·126	70–80	$26·56 \pm 4·41$
	3	2	14	45	3·241	5–10	$15·56 \pm 3·45$
	4	1	8	14	—	—	$28·57 \pm 6·61$
ad-4(44206)	1	7	10	150	12·307	5–10	$3·33 \pm 1·02$
	2	3	16	55	11·916	< 1	c. 14·6
	3	2	6	40	0·373	50–70	$7·50 \pm 2·82$
	4	3	4	56	0·403	80–90	$3·57 \pm 1·72$

All data from Houlahan *et al.* (1949).

* 1. = (A4 × L) × L; 2. = (A4 × L)⊕; 3. = [(A4 × L)⊕] × L; 4. = [(A4 × L) × L] × L; where A = Abbott, L = Lindegren and ⊕ = selfed.

crosses of four different complex wild-type ancestries compared with one another are heterogeneous only for *ad-4* which is nearer the centromere. Furthermore, only this locus shows heterogeneity within crosses of one given ancestry type. The seven crosses of type 1 ancestry are sharply divided into two groups, four crosses giving a centromere distance of 0·0 and three crosses giving a distance of $6·49 \pm 1·92$. A significant divergence is shown by crosses of type 2 ancestry, one cross giving a distance of 0·0 and the other two, in good agreement with one another, a distance of $22·22 \pm 4·14$. A possible explanation of this behaviour is that type 1 being 'backcrosses' and type 2 'selfings', segregation of the genetic factor(s) controlling different centromere distances of a locus had occurred.

(e) Conclusions

When the crosses are arranged according to wild-type ancestry, heterogeneity in centromere distances of loci in all linkage groups for which there is data is eliminated with few exceptions. Chilton crosses behave similarly to Lindegren but both differ from Abbott 4 and Abbott 12 crosses which also differ from one another. In general the loci show a similar pattern to *mt* with each wild ancestry type. However, loci distal from their centromere behave differently from proximal loci in crosses involving Abbott 4 and Lindegren ancestries. In addition some variation in the pattern of heterogeneity in centromere distance may occur between linkage groups.

Finally, segregation of the genetic factor(s) controlling heterogeneity in centromere distances of a locus appears possible.

6. SEGREGATION OF THE GENETIC FACTORS CONTROLLING DIFFERENT CENTROMERE DISTANCES OF A LOCUS

To investigate further the possible segregation of one or more factors controlling different centromere distances of a locus and at the same time to obtain data, which were lacking, on Abbott 12 × Lindegren backcrosses, Abbott 12*a* was crossed with Lindegren 1*A*. Ascus 9 showing first-division segregation for *mt* was chosen and cultures of spore pair 1 and pair 2 were each backcrossed to the Abbott parent, and pair 3 and pair 4 to the Lindegren parent. The data for the resulting centromere distances of *mt* are given in Table 8.

Comparatively immature, as well as mature, asci were dissected by the method described above so that any bias due to second-division asci maturing first would be minimized. The crosses with identical strains were repeated several times and consistent results were obtained, homogeneity of the crosses for each spore pair were all in the probability levels between 70 and 95%. The total sample sizes were increased to reduce errors due to small sample sizes. Records were kept of the number of non-germinating spore pairs and the number of asci which could not be scored for *mt*. These showed that high or low centromere distances resulted both with complete germination and analysis or with some failures. Hence viability differences were not causing bias.

The results (Table 8) indicate that there is a segregation in the ascus of one or more factors controlling significantly different centromere distances of *mt*, as if centromere distances were genetically determined (Frost, 1955 *b*).

Table 8. *Segregation of heterogeneous centromere distances of* mt *and* asco *in Ascus 9 arising from the cross Abbott 12*a × *Lindegren 1*A

Ascus 9 spore pair	2nd-div. asci	Total asci	Asci dissected	Centromere distance	2×2 χ^2	$P(\%)$
		× Abbott 12*a*.	Scored for *mt*			
1*A*	8	57	64	$7\cdot02 \pm 2\cdot30$		
2*A*	27	94	102	$14\cdot37 \pm 2\cdot33$	$4\cdot299$	2–5
		× Lindegren 1*A*.	Scored for *mt*			
3*a*	18	117	117	$7\cdot69 \pm 1\cdot67$		
4*a*	20	69	79	$14\cdot49 \pm 2\cdot73$	$4\cdot939$	2–5
		× *asco*(37402)*a*.	Scored for *asco*			
1*A*	81	347	—	$11\cdot67 \pm 1\cdot14$		
2*A*	121	362	—	$16\cdot72 \pm 1\cdot24$	$8\cdot840$	< 1

Furthermore, cultures of Ascus 9 spore pair 1 and pair 2 were each crossed to the same isolate of *asco* which is in linkage group VI. A segregation of the genetic factor(s) controlling significantly different centromere distances occurred also for

asco. The spore pair which gave the significantly lower distance for *mt* also gave the significantly lower distance for *asco* and vice versa. Hence two loci in different linkage groups show the same pattern of heterogeneity.

However, the same isolate of *asco* was crossed to some random spore progeny of pure Lindegren crosses, viz. $1A \times 25a$ and the backcross $(1A \times 25a) \times 1A$. Here the centromere distances of *asco* are homogeneous (Table 9). Comparing these data with

Table 9. *Centromere distance of* asco(*37402*)a *in crosses to Lindegren 1*A *and to random spores from 1*A $\times$ *25*a *and (1*A $\times$ *25*a*) $\times$ *1*A

Cross* $\times$	2nd-div. asci	Total asci	Centromere distance
L1	117	318	18·4
L1/L25–RS2	39	103	18·9
L1/L25–RS3	68	186	18·3
L1/L25–RS6	39	108	18·1
L1/L25–RS7	46	142	16·2
L1/L25–RS8	22	49	22·4
L1/L25/L1–RS3	45	130	17·3
L1/L25/L1–RS5	22	57	19·3
L1/L25/L1–RS6	33	83	19·9
L1/L25/L1–RS9	30	91	16·5
Total	461	1267	

Het. $\chi^2 = 3·802$, $n = 9$, $P = 0·90$–$0·95$.
Centromere distance of *asco* $= 18·19 \pm 0·68$.
* L1 = Lindegren $1A$; L25 = Lindegren $25a$; RS = random spore.

the Ascus $9 \times asco$ crosses in Table 8, then spore pair $1 \times asco$ shows a significant reduction over the Lindegren $1A \times asco$ distance ($\chi^2 = 14·355$, $n = 1$, $P = <0·01$) but spore pair $2 \times asco$ is not significantly different from the $1A \times asco$ distance. Unfortunately the wild ancestry of the *asco* strain used is unknown but presumably it is a re-isolate and hence the wild ancestry of these two crosses may be complex.

Combining the heterogeneous data for *mt* from spore pairs 1 and 2 which are crosses of (Abbott 12 × Lindegren) × Abbott 12 ancestry, a centromere distance of 11·3 for *mt* is obtained. This is in good agreement with the data (Table 2) for (Abbott 4 × Lindegren) × Abbott 4 crosses but perhaps through small sample sizes heterogeneity cannot be detected by the χ^2 test in the latter data but the graphical method of Mosteller and Turkey indicates some scatter.

Since E5297 arose from a random spore, it can be compared to spore pair 4 (Table 8) where the cross is of (Abbott 12 × Lindegren) × Lindegren ancestry. The centromere distance of *mt* here is in good agreement with that from E5297 × Lindegren crosses (Table 2) which are of identical wild ancestry. This suggests that segregation of genetic factor(s) controlling different centromere distances occurred in the spore which gave rise to E5297.

Since heterogeneity arises in the centromere distances of the same isolate of *asco* only when Abbott 12 is involved in the ancestry, it may be concluded that Abbott 12 is causing this effect. Although the analyses have been carried out only on one

ascus, it is apparent that at least one genetic factor must be carried by Abbott 12 resulting in a significantly increased centromere distance of *mt*. Furthermore, the comparison with the corresponding Abbott 4 data suggests that Abbott 4 too carries a similar factor or factors. Whether these factors in the two Abbott strains are identical is not apparent.

7. HETEROGENEITY IN RECOMBINATION FREQUENCIES BASED ON RANDOM SPORE ISOLATES

In random spore analyses, Frost (1955 *b*) found several cases of heterogeneity in recombination frequencies of which two will be reported here. A three-point test-cross of re-isolated strains of *arg-3* and *ad-3* gave in repulsion 3·2% recombination (3/93) between *arg* and *mt*, but one of the double mutants outcrossed to Lindegren I gave in coupling 15·8% recombination (12/76) in this interval. A comparison gave $2 \times 2\chi^2 = 8\cdot162$, $n = 1$, $P = <0\cdot01$. The other intervals were in good agreement. Germination was 76 and 78% respectively and progeny segregations were in good agreement with expectation. From the repulsion cross, 52 ordered asci (germination 78%) gave the order *mt, arg-3*, centromere, *ad-3*. However, an isolate of *arg-3* related to that used above was crossed to *al-2*. 43·3% recombination (45/104) occurred in the *arg* to *al* interval, 10·6% (11/104) between *arg* and *mt*, but 40·4% (42/104) between *al* and *mt*, thereby indicating a reversal in the order of *mt* and *arg* as if an inversion were involved. Germination was 77% and progeny segregations in agreement with expectation.

A second case involved a cross of *arg-6* with *nic-1* giving 5·6% recombination (11/197). Germination was 98%. A double-mutant from this cross was then crossed to *al-1* and 12·5% recombination (17/136) occurred between *arg* and *nic*. Germination was 85%. In both crosses progeny segregations were in agreement with expectation. The two recombination frequencies were significantly different ($\chi^2 = 4\cdot984$, $n = 1$, $P = 0\cdot02$–$0\cdot05$).

All the loci mentioned above are in linkage group I. Heterogeneity in centromere distances of loci distal from their centromere could arise by altered recombination frequencies proximal to the centromere only (Rifaat, 1958). These random spore analyses, however, indicate that heterogeneity can be present in recombination frequencies over short intervals distal from as well as proximal to the centromere.

De Serres (1958 *a*) found three significantly different groups of recombination frequencies between heteroalleles at the *ad-3* locus in crosses all involving only one wild ancestry, namely 74*A*. As shown in Table 1, however, 74*A* is a hybrid involving Abbott 4, Abbott 12 and Lindegren 1 and 25. It is of interest that three significantly different groups of values were obtained by De Serres as in the case of the centromere distances of *mt* reported above here where the same mixed wild ancestries are involved.

8. HETEROGENEOUS RECOMBINATION FREQUENCIES IN OTHER FUNGI

Heterogeneous recombination frequencies do not appear to be confined to *Neurospora crassa*. Fincham (1951) pointed out that the Arlington strain of *N. sitophila*

gave a lower centromere distance of *mt* than the English strain of this species. In addition, the data of Wülker (1935), which were derived from the second and third backcrosses to *N. sitophila* of an original cross of a strain of *N. sitophila* with a strain of *N. crassa*, gave a significantly reduced centromere distance of *mt* compared with the value from the English strain of *N. sitophila*.

Papazian (1950, 1951) found that when three wild strains from different localities of *Schizophyllum commune* were inbred, in two of the crosses recombination occurred in the *A* mating-type locus but the recombination frequency in these two crosses between *A* and *s* (streak) was significantly lower than in the remaining crosses in which no recombination occurred in the *A* locus. Here then an alteration occurred in both the frequency and distribution of recombination.

Dr J. R. Raper (personal communication) and Raper, Baxter & Middleton (1958) claimed that the frequencies of the origin of new mating-type alleles by recombination in their stocks of *Schizophyllum commune* showed significant differences between crosses involving different wild strains. The greatest divergence occurred when a wild strain from California and another from New Guinea were crossed with local wild strains from Illinois.

Day (1958) found heterogeneity in recombination frequencies in *Coprinus lagopus*. He suggested this might have arisen from the use of different wild strains in the crosses concerned.

In *Venturia inaequalis*, Boone & Keitt (1956) reported discrepancies in centromere distances and the occurrence of extra-chromosome progeny similar to pseudo-wild-types in *Neurospora*. Here again different wild strains may have been involved in the ancestry of the crosses.

In contrast all the mutant strains of the homothallic fungus *Aspergillus nidulans* arose or have been induced from a single wild-type strain (Pontecorvo, 1953). The extensive data obtained from these strains have revealed no case of heterogeneity in recombination frequencies.

In addition Whitehouse (1954), using strains of *Coprinus fimetarius* all of which were descended from a single wild fruit-body, failed to detect recombination in the incompatibility loci.

Frost (1955 *b*) suggested that the use of mixed wild ancestries instead of one might be experimentally convenient in significantly increasing and so detecting rare recombination between pseudo-alleles. The work of De Serres (1958 *a*) supports but does not conclusively prove this suggestion since in his crosses involving only one wild-type ancestry he used 74*A* which is a hybrid of several wild strains.

9. DISCUSSION

The experiments and data given above do not support the suggestions made by a number of workers that heterogeneity in recombination frequencies might arise from early maturation or preferential germination of certain asci or random spores; or from the presence of chromosome aberrations; or from irradiation effects additional to the induction of the biochemical mutation. The latter explanation

might be tenable for the exceptional behaviour of strain 27663 which gave significantly different centromere distances when two crosses both of Abbott 4 × Lindegren ancestry were compared and when opposite mating-types of 27663 were crossed to *ad-4*(44206) (data from Houlahan *et al.*, 1949).

On the other hand, the work of Singleton (1948), Tatum & Bell (1946), Holloway (1953), Garnjobst (1953), Hirsch (1954), Faull (1930) and Fincham (1950, 1951) indicates that the different wild strains of *N. crassa* show cytogenetical, physiological and ecological differences. In general these differences are of the same order, but not of the same magnitude, as the differences between *N. sitophila* and *N. crassa*. Thus compared to one another the wild strains of *N. crassa* are at least genically different or even carrying chromosome complements which are no longer strictly homologous.

Hence the genetical factors controlling recombination frequency might be the distinct genomes of Abbott 4, Abbott 12 and Lindegren wild-types. Since heterogeneity in recombination frequency can arise between heteroalleles, a locus and its centromere, or within short marked intervals proximal or distal to a centromere, the simplest explanation would be an alteration in effective pairing. Pontecorvo (1959) has suggested that heterozygocity might increase the frequency of exchanges in effectively paired segments. Thus the reduced frequencies in Abbott 4 × Lindegren crosses could be attributed to a lack of homology. As shown above, all other crosses of mixed Abbott and Lindegren ancestry result in unchanged or increased frequencies. However, in the first backcross to Abbott 4 of an Abbott 4 × Lindegren hybrid not only is homology restored but recombination frequencies may be increased. According to Perkins (1959), Stadler found a tendency for increased frequencies with successive backcrosses, i.e. homozygocity.

It would appear more likely, as Frost (1955 *a*) suggested, that the Abbott strains are roughly intermediate between the Lindegren strains of *N. crassa* and the New Zealand strains which are more closely allied to *N. sitophila* (Fincham, 1950). In his studies on the comparative maps of the mating-type chromosome of *N. crassa* and *N. sitophila*, Fincham (1951) stated that much of the difference could be explained by the degree to which the centromere interfered with crossing-over in its vicinity.

This interference could be strong in the Lindegren strains but far less so in the Abbott wild-types and might be under genetic control. The diploid ascus fusion nucleus of Abbott 4 and Lindegren might then intensify this interference for proximal loci. The backcrosses to Abbott 4 would show segregation of strong centromeric interference but also even increased recombination frequencies as shown for *ad-4* above. Possibly the genetic control could reside in the centromere itself.

However, centromeric interference could not explain all the differences since distal linked loci also show increased recombination frequencies. The Abbott strains must also carry genetic factors increasing detectable exchanges in effectively paired segments over the whole length of the chromosomes. It appears that these patterns of recombination are genetically controlled simultaneously in all the linkage groups but possibly with some variation between groups.

Such a hypothesis does not disagree with the claim by Stadler (1956 *a*) that many separable hereditary factors affected crossover frequency, or the claim of Rifaat (1958) that at least two genes with equal and cumulative effect controlled recombination frequencies in small regions adjacent to the centromere in linkage group I.

Barratt (1954) cautioned workers on the possible effects that Abbott stocks might have on mapping, interference and pseudo-allelic data. As far as interference studies are concerned, Howe (1956), Stadler (1956 *b*) and Perkins (1959) obtained results in conflict with those of Lindegren (as re-analysed by Whitehouse, 1948). It should be pointed out that the former workers used strains of mixed Abbott and Lindegren ancestry, unlike Lindegren, who used only the pure Lindegren wild and mutant strains. On the hypothesis above by which heterogeneity might arise, this conflict between the two sets of data is not unexpected.

Barratt (1954) suggested minimizing the effects of Abbott stocks by an intensive inbreeding programme. With several factors controlling recombination frequency this may not be effective. As shown above, the Lindegren wild strains give consistent, homogeneous results. Thus heterogeneity in recombination frequencies could be eliminated by repeatedly backcrossing all markers used to the Lindegren wild-type (Frost, 1955 *b*). This and other phenomena reported above are being investigated further.

SUMMARY

The available data on heterogeneity in centromere distances for a number of loci in several linkage groups are analysed and interpreted. When the crosses are grouped according to wild-type ancestry, heterogeneity is eliminated in any one group except those which consist of backcrosses or intercrosses. Abbott 4 and 12 are shown to be the source of the heterogeneity while Lindegren and probably Chilton wild strains give consistent, homogeneous distances. In a cross between Abbott 12 and Lindegren wild-types, the centromere distances of *mt* and *asco* show heterogeneous values between the spore pairs in an ascus indicating that significantly different distances are genetically determined and that the factors concerned show segregation. The genetic determination differs in the various wild strains; the data suggest that at least three factors are involved. In random spore analyses heterogeneity is present in recombination frequencies between linked markers either proximal or distal to their centromere. The mechanism by which heterogeneity in the data might arise is discussed. To obtain homogeneous data it is suggested that all markers used should be repeatedly backcrossed to the Lindegren wild-type.

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