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Antigenic polymorphism in a wild population of *Paramecium aurelia*

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1. INTRODUCTION

Antigenic polymorphism, as is well known, occurs in a wide variety of organisms, from bacteria to man, and *Paramecium* is no exception in this respect. Previous work (Beale, 1954; Pringle, 1956) has shown that a population of *P. aurelia* in a single isolated pond may contain individuals capable of forming a range of different types of immobilization antigen, and the range of antigens formed by a clone derived from one individual may differ from the range formed by another. Usually several representatives of alleles at each of several loci can be shown to be present in one population, if sufficient samples are taken. The mechanism whereby genes at different loci are brought to expression under control of the cytoplasm has been described in detail elsewhere (see Beale, 1957, for earlier references).

The evolutionary significance of this kind of polymorphism is by no means clear. It is possible, as often assumed, that heterozygotes containing two antigen-determining alleles have a selective advantage over the two corresponding homozygotes, and if true this would suggest a basis for the maintenance of two or more alleles in a population; but so far there is practically no direct evidence on this point. Alternatively, there may be no selective advantage of any particular antigen-determining gene or combination of genes and the polymorphism must be presumed to have arisen by mutation and chance survival of diverse types. The gene frequencies in the population would arrive at an equilibrium, depending on the mutation frequencies to and from the various alleles.

With the aim of collecting some basic information relevant to these questions, we have made a study of a single self-contained population of *P. aurelia* in Blackford Pond, Edinburgh, over a period of 6 years. Each year samples have been taken and the frequencies of various antigen-determining alleles determined.

2. MATERIAL AND METHODS

Before describing details of the actual methods, we give the following information on the biology of the organism (for further details, see Beale, 1954). Individuals of *P. aurelia* may be considered as diploid organisms, in that a single individual can contain at the most two allelic forms of a given gene. The macronucleus, however, contains many diploid sets of chromosomes. Reproduction is normally

by asexual fission, which may occur once (or slightly more) in 24 hours at 19° C., assuming excess food, but under the conditions of Blackford Pond, Edinburgh, the temperature is usually lower than this and the amount of food usually so small that the fission rate would be expected to be much less than once in 24 hours. Two kinds of sexual process occur: conjugation and autogamy. Conjugation takes place between two individuals of opposite mating type, and may or may not involve the union of diverse genotypes. During conjugation meiosis (and hence Mendelian segregation) occurs. The two ex-conjugants from a single pair are, however, genically identical, and contain one haploid set of genes from each conjugant. Autogamy involves meiosis and an internal fusion of two genically identical haploid nuclei. Thus a population of paramecia passing through autogamy may show Mendelian segregation, since a given clone may give rise after autogamy to diverse ex-autogamous animals, but all ex-autogamous clones are homozygous in respect of all their genes. Under laboratory conditions autogamy occurs at intervals of approximately sixty fissions in the particular stocks used in these experiments, with suitable nutrition, i.e. there is an interval of about sixty fissions between one autogamy and the next, or between conjugation and autogamy. Conjugation, however, can occur much more often, provided that the two opposite mating types are present and the cultures are dense enough for the animals to come into contact.

Nothing is known with certainty concerning the means whereby individuals of *P. aurelia* pass from one pond to another. Drought-resistant stages such as cysts are unknown, and it must therefore be assumed that such movement can only occur through chance carriage by birds, etc.

All the material to be described is classified according to the Sonneborn (1957) terminology as syngen 9. This term was introduced by Sonneborn in place of the older 'variety' to denote a group of individuals capable of conjugating *inter se*, of exchanging genes and giving rise to a substantial proportion of viable progeny. Syngen 9 is the predominant syngen of *P. aurelia* in Blackford Pond. A minority of individuals or this species belong to syngen 2, and in addition there are numerous specimens of other species, such as *P. caudatum* and *P. bursaria* (Pringle, 1956), in the pond.

Abundance of the material varied greatly at different times of the year. Collections were made between May and January, most abundantly in October and November. No animals were found in February, March or April. Presumably during the latter period nutritional conditions are so poor that only a very few paramecia survive.

Samples of water, together with dead leaves or other decaying vegetation, were taken into small glass vials (4 × 1 in.). After 2–4 days at room temperature the samples were examined and individual specimens of *P. aurelia* isolated into culture medium (baked lettuce infusion with *A. aerogenes*). Only a single clone of syngen 9—progeny of one wild paramecium—was taken from any one vial, thus eliminating complications due to fission in the vial of the originally collected organisms.

Table 1. *Numbers of clones containing various* G *and* X *alleles in collections made in successive years*

	G types				X types						X type unclassified
Season	G^1G^1	G^2G^2	G^1G^2	G^3G^3	X^1X^1	X^2X^2	X^3X^3	X^4X^4	X^5X^5	X^6X^6	
1953	2	0	0	0	2	0	0	0	0	0	0
1954	60	12	7	1	65	10	1	0	0	0	4
1955	91	9	2	0	82	0	0	9	7	1	3
1956	16	4	0	0	14	0	0	3	2	1	0
1957	3	0	0	0	1	0	0	2	0	0	0
1958 (E. end)	26	1	5	0	27	0	0	3	0	0	39
(W. end)	99	4	24	0	78	0	0	12	0	0	
Totals	297	30	38	1	269	10	1	29	9	2	46

Notes: (1) Previously (Pringle, 1956), the alleles were designated as follows: $G^1 = G^{510}$; $G^2 = G^{506}$; $G^3 = G^{509}$; $X^1 = X^{510}$; $X^2 = X^{530}$; $X^3 = X^{531}$; the remainder (X^4–X^6) were not recorded earlier.

(2) The season is denoted by the year in which most of the collections were made—i.e. '1954' collections were made from May 1954 till January 1955.

(3) The column 'X type unclassified' refers to cultures which died out before producing an X type antigen.

At various times after collection (sometimes as long as a year later), the G and X serotypes of every individual clone were determined by growing samples at 25° and 30° C. respectively and testing with appropriate antisera as previously described (Pringle, 1956). It should be mentioned that, in addition to the G and X types, a number of other serotypes are quite often found in syngen 9 of *P. aurelia*. When this occurs it is necessary to make repeated sub-cultures at the appropriate temperatures and where necessary screen out the unwanted types with specific antisera. Eventually every clone which did not die out during this treatment could be made to produce both G and X serotypes, i.e. there were no 'nuls' (see Beale, 1957).

3. RESULTS

The frequencies of the different G and X types are shown in Table 1. It will be noticed that heterozygotes appear amongst the G types but not amongst the X types. This is because the G types could usually be identified soon after isolation of animals from the vials, but a prolonged series of re-isolations was sometimes necessary before the X types could be obtained. During this time autogamy occurred and any heterozygotes which might have been present in the original collections would have given rise to one or other of the two possible homozygotes. In fact, X-type heterozygotes were found (e.g. X^1X^4), but to include these in Table 1 would give a misleading impression of their frequency.

For the same reason, the total numbers appearing amongst the X types are fewer than the G types, since, during the course of the long procedure described above, forty-six of the clones died out before they could be classified as regards their X type.

In Table 2 the data are combined so as to show the total numbers of individuals containing the various combinations of *G* and *X* alleles.

Table 2. *Total numbers of collected animals showing various combinations of* G *and* X *alleles*

	X^1X^1	X^4X^4	X^2X^2	X^5X^5	X^6X^6	X^3X^3	*X* alleles unclassified
G^1G^1	218	24	10	9	1	1	34
G^2G^2	26	2	0	0	1	0	1
G^1G^2	24	3	0	0	0	0	11
G^3G^3	1	0	0	0	0	0	0

4. DISCUSSION

Owing to the relatively small numbers of certain types in any given year, the data have been combined into two groups, the first for the first three seasons 1953–5, the second for 1956–8, as shown in Tables 3 and 4. Simple inspection of Table 3 is sufficient to show that there has been no change in the relative proportions of the three *G* alleles over the period of the observations. As regards the

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X types, shown in Table 4, it might appear that the frequency of X^{2*} has decreased and that of X^4 has increased, but taking the data as a whole there is no significant change ($\chi^2 = 3{\cdot}7$; $P = 0{\cdot}3$, as applied to the data for X^1, X^2, X^4, X^5).

Table 3. *Total numbers of* G *alleles found over two periods each of 3 years*

	G^1	G^2	G^3
1953–1955	315	51	1
1956–1958	317	47	0

Table 4. *Total numbers of* X *alleles found over two periods each of 3 years*

	X^1	X^2	X^3	X^4	X^5	X^6
1953–1955	149	10	1	9	7	1
1956–1958	120	0	0	20	2	1

In the last year (1958), samples were collected separately from the two ends of the pond (approximately 200 m. apart). As seen in Table 1, the proportions of G and X alleles, and of heterozygotes in relation to homozygotes, are similar at opposite ends of the pond.

It is therefore concluded that there are no grounds for disbelieving that the pond contains a homogeneous population, which has been stable in genic content for 6 years.

Considering now the various combinations of G and X alleles (Table 2), it is seen that the frequencies are such as would be expected by a random combination of the various alleles at the two loci. Here it should be mentioned that the G and X genes have previously been shown by laboratory breeding experiments to segregate independently (Pringle, 1956). Assuming that on first introduction of the species into the pond, only one or a very few individuals were present, these would then be expected to multiply clonally, and if gene recombination did not occur the population would come to contain disproportionately large numbers of certain genotypes, and few or none of others. Since the data in Table 2 show no evidence of such a disproportionality, it is inferred that conjugation, gene recombination and segregation have been occurring regularly. In any case, the existence of heterozygotes is in itself proof of the occurrence of conjugation in nature. Autogamy inevitably leads to complete homozygosis; therefore conjugation must take place if any appreciable frequency of heterozygotes is to be maintained.

Turning now to the relative numbers of heterozygotes and homozygotes at the G locus, the data are summarized in Table 5. The gene frequencies are: G^1, 0·87;

* There is a slight possibility that X^2 and X^4 are identical. Animals of type X^2 had all died out before they could be compared directly with X^4, but serum prepared against X^2 type was ineffective in immobilizing X^4 animals.

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G^2, 0·13; G^3, 0·004. Assuming random conjugation amongst the three types G^1G^1, G^1G^2, G^2G^2, no autogamy, and no selection of any type, the expected numbers have been calculated and are shown in Table 5. There is a large deficit of heterozygotes, which is not surprising, of course, in view of the known occurrence of autogamy. Nothing is known about the relative frequencies of conjugation and

Table 5. *Numbers of heterozygotes and homozygotes at the* G *locus*

	G^1G^1	G^2G^2	G^1G^2
Obtained	297	30	38
Calculated (see Text)	277·4	7·3	80·3

autogamy in nature, but considering the sparseness of the population opportunities for conjugation would seem to be few and one would then expect few or no individuals to be heterozygotes.

However, it must be admitted we do not know to what extent conjugation occurs under the nutritional and other conditions existing in nature, nor whether there is any significant conjugational inbreeding amongst the offspring of particular conjugating pairs. These topics are discussed at length by Sonneborn (1957).

Kimball *et al.* (1957) found in certain laboratory (test-tube) populations that conjugation was the predominant sexual process and autogamy comparatively rare, but the conditions were there highly artificial since there was an abundance of food, the cultures were dense, and meetings between animals of opposite mating types would occur frequently.

A factor which would counteract the effect of autogamy in maintaining a high proportion of homozygotes would be natural selection of heterozygotes, should such a phenomenon exist, but there is at present no information on whether such selection does in fact operate in this system. Siegel (1958) found evidence that some hybrids had a higher fission rate than pure stocks, but the comparison here involved quite distinct stocks originally isolated from widely separated natural populations, which would differ in many genes. His results are therefore not comparable with ours.

All the data on heterozygotes obtained in our experiments are concerned with the G locus. It is worth noting here that tests directly made on animals immediately after collection from Blackford Pond show that the G serotype is the one normally produced in nature in this population. There would thus be ample opportunity for natural selection to operate on the heterozygotes G^1G^2 containing a mixture of the two G-type antigens. With the X alleles, however, the situation is quite different. The antigens controlled by these genes are only formed in paramecia growing at temperatures above 28°C., which are never reached in Blackford Pond. There would therefore be no opportunity for selection to operate on

the immobilization antigens produced by any of the X alleles, and it follows that only the relative frequency of conjugation and autogamy would control the proportion of heterozygotes at this locus. (This disregards the possibility that the antigen-determining genes affect, in addition to the specificity of the immobilization antigens, other unknown properties of the organisms.)

It is to be hoped that in the future estimates will be made of the proportions of heterozygotes for alleles, such as the X alleles, which are not expressed in nature. Assuming then an absence of selection, the discrepancy between expected and obtained proportions of heterozygotes would give an indication of the importance of autogamy. It would then be possible, by comparing such results with the proportions of heterozygotes for genes which are expressed in nature, such as the G alleles, to discover whether there was any effect of natural selection of the latter.

SUMMARY

1. A population of *Paramecium aurelia*, syngen 9, in Blackford Pond, Edinburgh, has been sampled over a period of 6 years and the types of immobilization antigen present identified.

2. Three antigen-determining alleles at the G locus and six alleles at the X locus were found.

3. No significant changes in relative numbers of the various alleles were apparent over the period studied, nor was there any difference between opposite ends of the pond.

4. An appreciable proportion of G-type heterozygotes was found, but the numbers were significantly less than would be expected by random mating alone. Autogamy is presumed to be responsible for this deficit.

5. Whether there is any natural selection in favour of heterozygotes over homozygotes in unknown. A possible method for obtaining information on this subject is discussed.

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