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Stability and variation in aphid clonal lineages

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Evidence in the literature purporting to show genetic variability within parthenogenetic lines of aphids is reviewed. The results of three experimental approaches to this problem are reported: (1) a long term study of fluctuation in alate morph production in six lines of *Myzus persicae;* (2) an attempt tp select for caudal hair form in *Acyrthosiphon pisum;* (3) a study of the inheritance of esterase variants of *M. persicae* in sexual and parthenogenetic reproduction. It is concluded that genetic recombination during parthenogenesis is not the general rule in these aphids, and that aphid parthenogenesis should be regarded as of ameiotic or apomictic type. Possible alternative explanations for the different kinds of variation or apparent variation which occur within aphid clonal lineages are discussed.

KEY WORDS: – aphid – apomixis – endomeiosis – parthenogenesis – recombination – mutation – variation – polymorphism – position effect – *Myzus persicae* – *Acyrthosiphon pisum.*

CONTENTS

INTRODUCTION AND HISTORICAL PERSPECTIVE

It is over 230 years since Charles Bonnet first demonstrated the phenomenon of virgin birth in plant-lice (Bonnet 1745). Yet although we know that

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parthenogenetic reproduction enables aphids to exploit short-term food supplies and thus acquire considerable importance as agricultural pests, we still do not know how to treat the phenomenon of aphid parthenogenesis in genetic terms. "What is an aphid?" is a question recently posed by Janzen (1977). More specifically, what is the genotypic composition of an aphid population, and how does it respond and adapt to changes in the environment in the short term, or in the long term in the case of those many pest species which do not regularly produce sexuales. Basic to the whole problem of aphid population genetics is the nature of aphid clones. Yet there is still uncertainty even as to whether aphid parthenogenetic lineages are clones at all in the accepted sense of the term; that is, whether they consist of individuals which are all of identical genotype. This is a point which must be clarified before there can be any meaningful studies of the genetics of aphid populations.

Much of this uncertainty stems from a series of publications by Cognetti (1961a, b, 1962) and his co-workers (Cognetti & Dallari, 1961; Cognetti & Cognetti-Varriale, 1961; Cognetti & Pagliai, 1962, 1963; Boschetti, 1963; Boschetti & Pagliai, 1964; Pagliai, 1961, 1962, 1963, 1965, 1967a, b; Orlando 1965) in which results of cytological and experimental work on various aphid species are interpreted as demonstrating genetic recombination within parthenogenetic lines. The cytological work centred on the behaviour of chromosomes in the pre-growth phase oocytes in the germarium of the parthenogenetic female aphid. The observations of Cognetti's school in fact agreed to some extent with those of von Baehr (1920) and Paspaleff (1929) who reported pairing of homologous chromosones in early prophase followed by a condensation phase in which the homologues fell apart so that the diploid number was observable prior to the growth phase of the oocyte. Cognetti, however, proposed that crossing over occurred between homologues while they were paired, and chiasmata were formed which nevertheless did not prevent the homologues from separating again without even the dissolution of the nuclear membrane. He called this unique phenomenon 'endomeiosis'. This interpretation has been criticized by Suomalainen *et al.* (1976), who noted that none of the figures presented as evidence by Cognetti showed any clear chiasmata. Orlando & Mari (1968) and Blackman (1978) did not observe any clear diakinesis figures in parthenogenetic oocytes of four of the aphid species previously studied by Cognetti's school, and found that the first one or two oocytes to develop in each germarium did not even undergo condensation of their chromosomes prior to the growth phase.

During the maturation of parthenogenetic eggs of *Daphnia* the chromosomes behave in somewhat similar fashion, and Bacci, Cognetti & Vacari (1961) interpreted the transient pairing of homologues here also as a mechanism of genetic recombination. However, Hebert & Ward (1972) compared the enzyme variation in sexual and parthenogenetic lines of *Daphnia magna* and demonstrated that no recombination occurred at three different enzyme loci during parthenogenesis.

Proper genetic markers have hitherto been lacking in aphids, so that critical genetic experiments to determine whether or not genetic recombination occurs within parthenogenetic lines have not been possible. However, some evidence can be gleaned from the literature suggesting that recombination is not a regular feature of aphid parthenogenesis. For example, Müller (1962a) studied the inheritance of colour in *Acyrthosiphon pisum* and concluded that red was dominant

to green, yet apparently heterozygous (red) clones were not observed to become homozygous for the green allele (Müller 1962b, 1971a). Red-green colour polymorphism is a common feature of Aphidinae, and although the inheritance of colour has not been studied in other species it seems significant that there are very few reports in the literature of irreversible colour changes within clones. Recently, Baker (1978) reported that he could not detect recombination at an esterase locus (ESE) during parthenogenesis of *Myzus persicae,* but he had no evidence of the genetic relationships between the esterase variants involved.

Other experimental work has attempted to show whether it is possible to select for certain traits, which are assumed to be polygenically determined, within parthenogenetic lines. Ewing (1916) reared *Aphis avenae (=Rhopalosiphum padi)* through 87 parthenogenetic generations during which he tried to shift the mean values for six different biometric characters by selection of extreme variants, with absolutely no effects. However, Cognetti (1961), Cognetti & Dallari (1961) and Cognetti & Pagliai (1962) carried out selection experiments with *M. persicae* and *Brevicoryne brassicae,* in which the character selected for was the ability to produce apterous or alate progeny. Data were presented which indicated some reduction in the numbers of alate offspring produced in lines selected for 'apterousness'. The choice of a character which is so subject to the influence of environmental factors, and the lack of control of these factors during the selection procedure, were criticized by Lees (1966). In a more controlled experiment, Lees selected for alata-producers in a clone of *Megoura vicae.* The selection process was continued for 18 generations and had no effect on the percentage of individuals which produced alatae.

Cognetti (1962) and Cognetti & Pagliai (1963) also reported successful attempts to select artificially for the parthenogenetic morphs of *B. brassicae* in conditions favouring the production of sexual morphs. Some of this work can be discounted, as pointed out by Lees (1966), because it was conducted with populations of mixed genotypic composition, but in one case (Cognetti, 1962) the selections were reported to be made in a line started from a single fundatrix.

Pagliai (1967b) subsequently made selections for a morphological character in *Acyrthosiphon pisum.* This character, which was previously studied in some detail by Meier (1964) and shown to vary within as well as between clones of *A. pisum,* concerned the number of abnormal hairs on the cauda, recorded in terms of a "caudal hair alteration index". The results presented by Pagliai appear to show a convincing two-way selection for more and less abnormal caudal hairs, in three different clones of *A. pisum.* However, several aspects of this work suggest that the results should be treated circumspectly. First, the genetic determination of abnormal caudal hairs in *A. pisum* is unknown, and their occurrence could presumably be affected by factors in the prenatal or postnatal environment. Second, assessment of the caudal hair alteration index involves a subjective element as it requires different types of altered caudal hairs to be allocated to different classes. Third, the photoperiod conditions under which the experiments were conducted are not stated, but in two of the clones sexual females were obtained after eight generations, indicating that the photoperiod was not constant and could therefore have been invoking morphological changes within clones throughout the experiment. Fourth, in all three clones females selected for an increased number of abnormal hairs showed lower fertility and higher mortality than those selected for normal hairs. It is difficult to understand why

females with many abnormal hairs should be so common if they can be selected against within parthenogenetic lines.

Selection experiments performed within parthenogenetic lines of aphids are open to general objections which arise from certain physiological considerations peculiar to aphids. A parthenogenetic female aphid has a direct influence on the morphology of not only her daughters but also her grand-daughters, some of which start their embryonic development even before their mothers are born. Long-term timing mechanisms ("interval timers"; Lees, 1966), for which there are as yet no physiological explanations, can extend this influence to affect the morph of individuals within a clone over many generations. Therefore not only does the environment have to be strictly controlled during the course of a selection experiment, but for a long time beforehand. One of the principal factors affecting aphid morphology is in any case nutrition, which cannot be properly controlled unless the aphids are kept on artificial diets.

It is thus true to say that very little of the evidence put forward to support the existence of a mechanism of genetic recombination within aphid clones, such as Cognetti's 'endomeiosis', stands up to critical examination. However, there are numerous reports in the literature of variation and changes within parthenogenetic lines of aphids which are not clearly related to environmental factors, and these require explanation. Such reports become more frequent as aphid clones are more intensively studied. The two most frequently reported types of 'intraclonal' change are the loss of ability to produce sexuales (e.g. Bonnemaison, 1951; Cognetti, 1962; Müller, 1954; Ossiannilson, 1959), and the loss of resistance to organophosphorus insecticides (e.g. Dunn & Kempton, 1966; Hůrková, 1970; Beranek, 1974a). In particular, 'endomeiosis' has been proposed as the explanation of loss of resistance with such regularity (Helle, 1968; Hrdý *et al.*, 1970; Rassmann, 1973; Boness & Unterstenhöfer, 1974) that it is in danger of becoming accepted as fact.

The purpose of the present paper is to report new evidence for the general genetic stability of aphid parthenogenetic lineages in both the short and the long term, using three different experimental approaches. The discussion will then centre on possible alternative explanations for those cases reported in the literature which provide evidence for intraclonal variability, but which cannot be explained in terms of known effects of the environment on the phenotype.

LONG-TERM STUDIES OF ALATE MORPH PRODUCTION IN CLONES OF *MYZUS PERSICAE*

Introduction

Wing polymorphism has been intensively studied in aphids, and *M. persicae* was one of the species in which the role of crowding in promoting the development of the alate morph was first demonstrated by Bonnemaison (1951). Since Bonnemaison's work a number of other factors beside crowding have been implicated in alata determination in *M. persicae*. In particular, work with artificial diets has shown that the "switch mechanism" diverting embryos or larvae to the apterous or the alate developmental pathway is sensitive to various nutritional factors (Harrewijn, 1976; Mittler, 1973). However, the interaction between the direct influence of a dietary deficiency on wing polymorphism, and its indirect effect by inducing restlessness and hence increasing the intensity of the crowding stimulus, still causes problems in the interpretation of results.

Although wing polymorphism is very difficult to study as a genetic character, because of this complex environmental component of its expression, it must of course be ultimately under genetic control. In the course of long-term rearing of clones of *M. persicae* in relatively controlled conditions, it has been possible to accumulate data on the numbers of alatae and apterae among the first-born progeny of a great many successive generations (up to 170). Because of its long-term nature this data overcomes the problem of short-term fluctuations due to insufficient control over environmetal influences such as nutrition, as well as the maternal factors whereby one generation can influence the morph of the next. In the long run the effects of the environment cancel each other out revealing the underlying genetic component of wing polymorphism.

Method

Clones of *M. persicae* started from single apterous virginoparae from a variety of sources were reared on excised leaves of potato (var. Majestic) in small perspex boxes at 18°C and 16-hr photoperiod. To guard against contamination, all potato plants were regularly fumigated with nicotine, and rearing boxes were only opened one at a time on a well-illuminated white glass working surface, which was wiped clean before handling each clone. Each generation was kept distinct from the previous one by rearing 25 new-born larvae synchronously to the adult stage and discarding them after they had produced the first 25 progeny of the next generation. Thus the rearing density was maintained at 25 aphids per leaf. The numbers of alatae and apterae in the first-born of each generation were recorded when they reached the fourth instar. Larval development normally took about seven days for apterae, but nearly eight days for alatae, and the pre-larviposition period was also shorter for apterae than for alatae. Therefore unless very few apterae were produced in any one generation the first-born 25 progeny were invariably the progeny of apterae, and they were usually born even before the alatae had become adult. Thus, although there was no deliberate attempt to select for alata-producers or aptera-producers, this rearing procedure had a clear tendency to favour apterae rather than alatae, and in the long term it would be expected to decrease the proportion of alata-producers in a clone, if this were possible.

Results

Figure 1 shows that in up to 170 generation of parthenogenetic reproduction by *M. persicae,* there was no significant change in the overall incidence of alate morph production by six different clones. Instead, it can be seen that, while the short term fluctuations from generation to generation within each clone can be extreme, each clone maintained a consistent, overall level of alata production. This no doubt reflects the underlying genetic component of the determination process, and when recorded over a sufficient number of generations it is a stable characteristic of the genotype of that clone.

Further examination of the data in Fig. 1 can serve to emphasize the misleading conclusions that could be drawn from the results of relatively short-term experiments. For example, the clone 'Versailles', after producing alatae for several generations when records started at the end of 1973, produced no

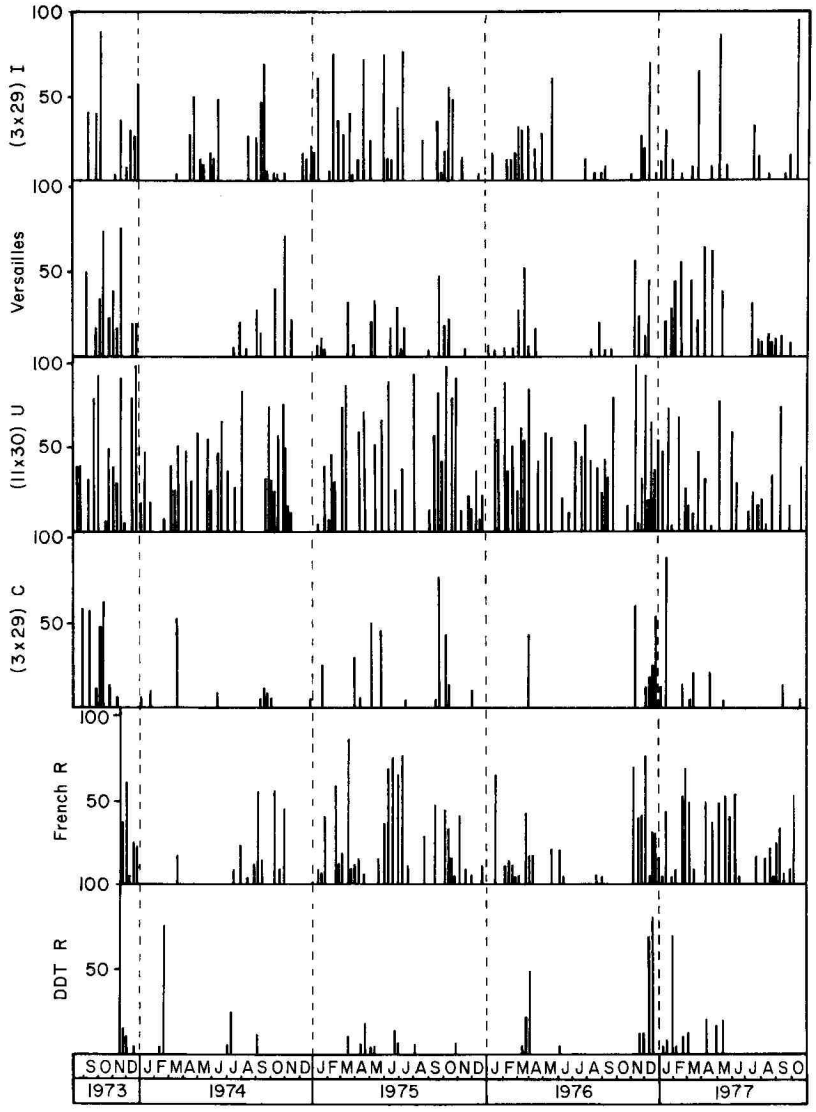

Figure 1. The percentage of alatae among the first-born progeny in six clones of *Myzus persicae* reared on excised leaves of potato at 16 hrs and 18°C, from August 1973 to October 1977.

alatae at all until half-way through 1974 (a span of 18 consecutive generations), yet over 170 generations a consistent pattern emerged for the incidence of the alate morph within this clone. Alata production was also at a relatively low ebb in most other clones during the first half of 1974. The fluctuations were, in fact, not entirely random, for there was a tendency for peaks of alata-production to coincide in different clones; for example, in September/October 1975 and in November 1976 to January 1977 (Fig. 1). The common factor for all six clones which it was not possible to control adequately was nutrition. It seems likely that variations in the food quality of the excised leaves, resulting either from

environmentally or genetically-determined differences in the plants from which they were taken, were responsible for those fluctuations in the level of alata-production which extended over several generations. Extreme differences from one generation to the next, which were frequent in the clones which produced more alatae, were probably due to maternal effects. Where 100% alatae were obtained, which only happened on four occasions, the first-born of the next generation were invariably all apterae. Likewise, 90% or more alatae in one generation was never followed by more than 20% in the next, and no two successive generations were ever obtained with more than 80% alatae.

SELECTION FOR 'CAUDAL HAIR ALTERATION INDEX' IN A CLONE OF *ACYRTHOSIPHON PISUM*

Introduction

Although open to criticism on several grounds, the results of Pagliai (1976b) in selecting for normal and altered caudal hairs in three separate lines of *A. pisum,* seem to provide the most convincing evidence available for the existence of some form of genetic recombination during aphid parthenogenesis. It was therefore decided to repeat Pagliai's selection experiment to see if similar results could be obtained.

Method

A green clone of *A. pisum* was used which had been reared continuously under conditions favouring parthenogenesis for over five years. The aphids were maintained at 18°C and 16-hr photoperiod throughout the course of the experiment. The precedure followed was based on that of Pagliai (1967b). A single young adult apterous virginopara was isolated on an excised leaf of *Vicia faba* (var. "The Sutton"). After one week of larviposition, this aphid was sacrificed and mounted on a slide, and its caudal hair alteration index was determined (Meier, 1964). Upon reaching maturity, 20 of the daughters of this first individual were isolated on excised bean leaves in separate cages. One week after the start of larviposition, the alteration indices of these 20 females were calculated, and the female with the highest index was chosen as the founder of line H, selected for altered caudal hairs, while the female with the lowest index was chosen as the founder of line L, selected for normal caudal hairs. Thereafter in successive generations line H was continued using progeny of the female with the highest index, and line L was continued using the progeny of the female with the lowest index.

In order to eliminate the possibility of bias in the assessment of the caudal hair alteration index, the slide-mounted adults from both high and low-selected lines were pooled for each generation and allotted code numbers, so that the person estimating the index did not know which aphids were from which line.

Cytological studies have shown that the development of the first oocyte in each ovariole in a parthenogenetic female of *A. pisum* differs radically from that of subsequent oocytes (Blackman, 1978). The chromosome condensation phase, which was interpreted by Cognetti as a stage in endomeiosis, is lacking in the first oocyte and is only observed in the remaining oocytes at the base of the germarium after the first oocyte has been ovulated. Thus the first-born of each generation

should perhaps be discarded in selection experiment as atypical, and the selection made from later-born progeny which had developed from oocytes that had undergone the condensation phase. Pagliai used progeny born during the first week of larviposition for her experiments. Nevertheless, to make sure that the manner of oocyte development had no effect on the selection results, in generations 7–14 both H and L lines were maintained using only progeny born after the first week of larviposition, the first-born progeny being discarded.

Results

In complete contrast to the results reported by Pagliai, there was no evidence at all of any divergence in the values of the caudal hair alteration index between high and low-selected lines (Fig. 2). In the first generation of selection, adults of line H (high-selected) had a mean index of 0.60 (±0.05), and adults of line L (low-selected) had a mean index of 0.69 (±0.05). Despite some fluctuation in value in subsequent generations, the values obtained in the last (14th) generation of selection were 0.68 (± 0.03) for line H and 0.69 (±0.05) for line L. In the intervening generations the mean index for line L was as often above that of line H as below it, and in the only generation (no. 3) that there was a significant difference between the two lines, it was line L which had the higher index.

The mean value for the index for all individuals combined was 0.72, which is approximately in the centre of the range of values given by Meier (1964) for ten clones of *A. pisum*. Values for individual aphids within the clone varied from 0.33 to 1.08, which is almost exactly the range of variation reported by Meier within his clone 5 (mean index 0.74). Therefore assuming a polygenic determination of the index there is no reason why its value should not have been raised or lowered by selection, if recombination could occur within the clone.

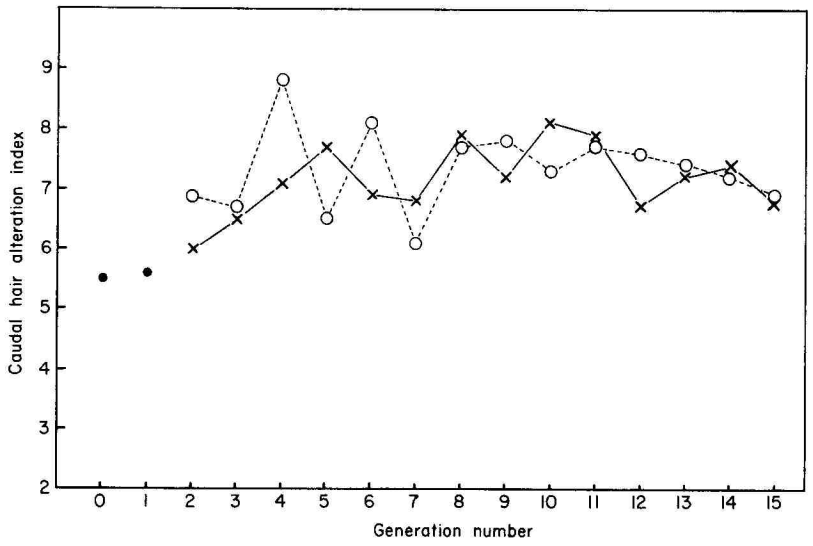

Figure 2. Fluctuations in the mean value of the "caudal hair alteration index" of *Acyrthosiphon pisum* during attempts to select for high (x) and low (O) values of the index. Both high and low-selected lines originated from a single aphid at generation 0: the index of this aphid, and the mean value of the index for her first generation progeny from which selection started, are indicated (●).

To investigate maternal effects, each parent aphid was classified according to its caudal hair alteration index, and the mean value for all the progeny of each class of parent was calculated. Using a method of illustration devised by Ewing (1917), it can be seen that the index of a parent had no significant effect on that of its progeny (Fig. 3). In many cases the progeny mean for a particular parental class 'overshot' the overall mean for the clone (0.72), although the general impression was of progeny means fluctuating randomly about the overall mean and completely independent of the parental index.

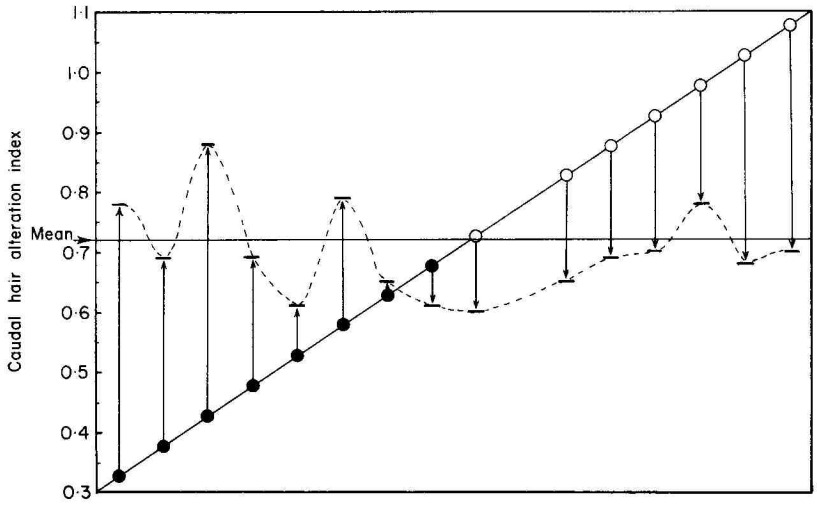

Figure 3. The relationship between the caudal hair alteration index of a parent aphid and that of its progeny. The parents are classified according to their index (0.30–0.34, 0.35–0.39, etc.), and each class is represented by a circle on the diagonal. Open circles are all parent of high-selected lines and closed circles are all parents of low-selected lines. The mean value of the index for all progeny of the parents in each class is indicated by a horizontal bar. The horizontal line indicates the mean value of the index for all progeny of all glasses of parent.

INHERITANCE OF ESTERASE VARIANTS DURING PARTHENOGENETIC AND SEXUAL REPRODUCTION OF *MYZUS PERSICAE*

Introduction

Conclusive proof of recombination within parthenogenetic lines of aphids can only be obtained by demonstrating the segregation of properly studied genetic markers. Recent studies of esterase variation in *M. persicae,* particularly in relation to the inheritance of resistance to organophosphorus insecticides (Blackman *et al.,* 1977, Blackman & Devonshire, in press), have revealed two types of electrophoretic variant which can be studied in this way.

Method

Enzymes were separated on horizontal starch gels (10%) using electrophoretic techniques described by Berenek (1974b). The gels were stained for esterase using Fast Blue BB salt with 1-naphthyl acetate as substrate. Genetic relationships between variants were established by crossing males and sexual females from

clones of known electrophoretic phenotype. The sexual morphs were produced by exposure to short photoperiod (Blackman 1972) and fertilized eggs were overwintered either on peach (*Prunus persica*) or with radish plants (*Raphanus sativus*) in insectary cages (Blackman *et al.*, 1978).

Results

The first type of esterase variation investigated was a polymorphism at the *est-1* (=ESE of Baker, 1977) locus. Clones of *M. persicae* were found to have one of three different forms of enzyme variant at this locus: a common, slow-moving form; a rarer, faster-moving form; and a third, common variant with a broader, more diffuse band of intermediate mobility (Fig. 4). Observed phenotype ratios in the sexual crosses were in agreement with those expected if the band of intermediate mobility represents the heterozygote, *sf*, of alleles *s* and *f* coding respectively for fast and slow enzyme variants (Table 1).

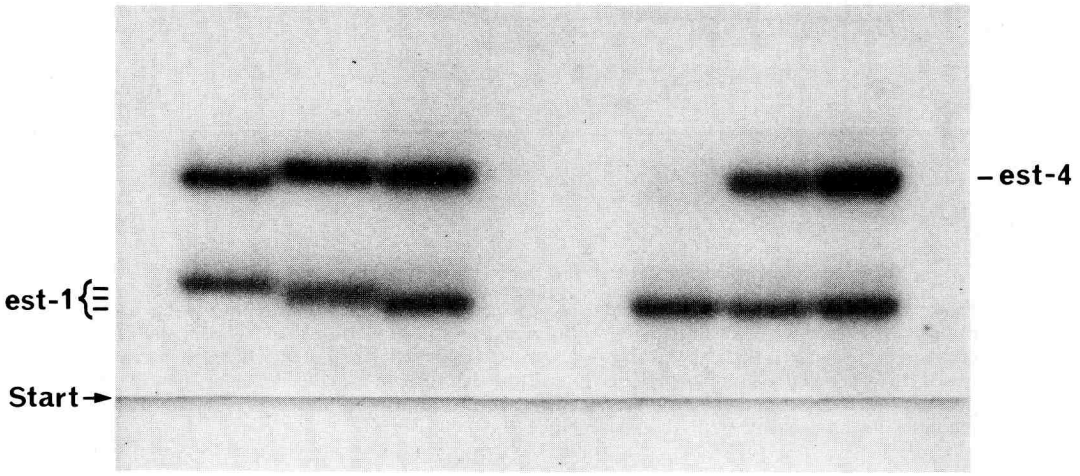

Figure 4. Electrophoretogram showing the esterase variants of *Myzus persicae*. The three samples on the left show the polymorphism at the est-1 locus: from left to right, ff, sf and ss. The three samples on the right show polymorphism in the regulator of est-4: from left to right, rr, Rr and RR.

Table 1. Inheritance of esterase variants in sexual reproduction of *Myzus persicae*

Locus	Cross	Number of F_1 clones	F_1 phenotypes	Fit to expected frequencies (χ^2)
est-1	sf × sf	37	6ff : 24sf : 7ss	$P>0.32$ (2.76)
	sf × ss	39	0ff: 17sf: 22ss	$P>0.3$ (0.69)
	ss × ss	91	0ff: 0sf: 91ss	—
est-4	Rr × Rr	91	24RR : 52Rr : 15rr	$P>0.1$ (3.64)

The second type of esterase variation investigated was at the *est-4* (=RAE of Baker, 1977) locus, and concerned a polymorphism, not of the structural gene itself, but of a regulator of this gene's activity. Increased activity of *est-4* is closely correlated in *M. persicae* with resistance to organophosphorus insecticides

Table 2. Inheritance of esterase variants in parthenogenetic reproduction of *Myzus persicae*

Locus	Maternal phenotype	Number of clones	Number of progeny examined	Progeny phenotype
est-1	sf	3	107	0ff : 107sf : 0ss
est-4 regulator	Rr	2	104	0RR : 104Rr : 0rr

(Blackman *et al.*, 1977). Although other loci seem to be implicated in high levels of resistance (Blackman & Devonshire, in preparation), sexual crosses indicate that the lowest level of resistance is due to a mutation at a single regulator locus (*r*) to give a form (*R*) with increased *est-4* activity (Table 1, and Blackman & Devonshire, in press). On starch gels, phenotypes of known parentage can be classified by eye, the heterozygote (*Rr*) having an *est-4* band of about equal intensity to that of *est-1*, and the homozygote for the mutant form of the regulator (*RR*) having an *est-4* band of about twice the density of *est-1* (Fig. 4).

If recombination occurred during parthenogenetic reproduction, one would expect segregation of variants at these two loci in the progeny of heterozygous parthenogenetic females, so that some progeny homozygous for each variant would be obtained. This clearly did not occur (Table 2). The progeny of parthenogenetic females heterozygous for *est-1* variants (*sf*) were all heterozygous confirming the result of Baker (1978), and the progeny of females heterozygous at the regulator locus for *est-4* (*Rr*) were also all heterozygous. There was therefore no evidence of any recombination affecting either of these loci.

DISCUSSION

The purpose of this work was not to show whether genetic recombination *ever* occurs within parthenogenetic lines of aphids, but to see if it occurs as a general rule, providing a significant and continual source of genetic variation, as claimed by Cognetti (1961). The results reported here for *Myzus persicae* and *Acyrthosiphon pisum,* which were the two species principally used by Cognetti and Pagliai for their cytological and experimental studies, indicate that genetic recombination during parthenogenesis is not the general rule in these species. In the absence of conclusive evidence to the contrary, parthenogenesis in aphids should therefore be considered to be one of the ameiotic or apomictic type.

This leaves the problem of providing an explanation for that variation which does occur, or has been reported to occur, within clones of aphids. Progress in genetic research in the last 20 years, especially in relation to the control of gene expression, has now opened the way to a number of alternative explanations based on known genetic phenomena in other organisms. It seems opportune to summarize the possible causes of variation, or apparent variation, in aphid clonal lineages, and discuss them in relation to the extensive literature on this subject.

Contamination of laboratory clones

It seem likely that the standards of hygiene needed to avoid contamination of laboratory clones with alien aphids, either from other clones reared in the same

laboratory or from outside sources, have frequently been underestimated by aphid workers. First instar aphids are very easily overlooked, especially on whole plants where they may be concealed in the growing point and will occasionally survive spraying or fumigation. One first instar intruder in the right circumstances may take over a culture. The risk of contamination is greatly reduced if excised leaves, leaf discs or sachets of artificial diet are used, and reduced still further if other precautions are taken, including careful control of numbers, synchronized rearing, and proper standards of hygiene. Nevertheless, it seems advisable to work with the premise that the risk of contamination can never be completely eliminated, and always to consider this possibility when assessing the results of experiments which appear to show changes within laboratory clones.

Shaposhnikov (1965) reared a clone of *Dysaphis anthrisci majkopica* on a relatively unsuitable host-plant (*Chaerophyllum bulbosum*) for eight generations. In the eighth generation, he noted that morphological changes had occurred in a proportion of the aphids, and after this he was able to continue the culture successfully on another species of *Chaerophyllum, C. maculatum,* which had previously been totally unacceptable. From the ninth generation the aphids on *C. maculatum* were remarkably similar to *Dysaphis chaerophyllina,* a species whose normal host is *C. maculatum.* When sexuales were produced and crosses made (Shaposhnikov, 1966), it was found that the 'new form' failed to produce any viable progeny when crossed with *majkopica.* However, crosses between the 'new form' and *chaerophyllina* were generally as successful as intraspecific matings. Shaposhnikov (1978) has discussed and interpreted these results as an example of a quantum shift in the evolution of this species-group giving rise to a new form of species rank. Morphological convergence of the new form with *chaerophyllina* is ascribed to their common host-plant.

The data presented by Shaposhnikov (1965) for the morphological transformations are sufficiently detailed to enable his results to be critically assessed. An alternative explanation can be put forward. Until the eighth generation, the culture on *C. bulbosum* was a clone of *majkopica.* During the eighth generation, the culture became contaminated with a clone of *chaerophyllina,* and subsequent generations after transfer to *C. maculatum* consisted entirely of this species. The rearing procedures used in this work (Shaposhnikov, 1959) clearly do not rule out the possibility of such contamination. The morphological differences found between the new form and *chaerophyllina* are in very variable characters, and are not so large as to be unlikely between clones within a species. In view of the results subsequently obtained in hybridization experiments, contamination must surely be regarded as the likeliest explanation of the changes observed.

The chances of contamination clearly mount up, the longer a clone is kept. Some of the reports of loss of ability to produce sexual morphs in old clones, especially of *Myzus persicae* (Bonnemaison, 1951; Müller, 1954; Ossiannilsson, 1959), could be due to this accumulation of chances. Contrary evidence of stability is provided by Waldhauer (1957), Lees (1966), Blackman (1971) and Müller (1971b), who kept holocyclic clones of various aphid species, including *M. persicae,* for 2 to 15 years without them losing their powers of sexual reproduction.

Mutations

A mutation in a gem-line cell of a parthenogenetic aphid will cause one or more progeny to differ in genotype from their mother, and thus have the

potential to found a new clone. Viable mutations are rare events, and even those having obvious phenotypic effects in the heterozygous condition are unlikely to be detected in laboratory cultures unless large numbers of aphids are reared and carefully scrutinized. It is somewhat surprising that no one seems to have conducted a systematic search for mutants in any one species of aphid.

However, field populations of aphids, especially those of pest aphids, are so large that even the rarest mutation is likely to occur many times (Dickson, 1962), and if it confers a significant improvement in fitness then the frequency of the mutant genotype will increase rapidly. It is even possible, given the size of aphid populations, that homozygous mutations and favourable combinations of mutations involving more than one locus could occur to a significant extent, offsetting the lack of genetic recombination. Recombination could in fact be a disadvantage in such circumstances, diluting the effect of the mutant allele and delaying its establishment in the population. There is growing evidence that populations of apomictic animals and plants often have considerable genetic variability and short-term adaptive potential (Suomalainen *et al.*, 1976). Genetic changes which result in immediate and very considerable increases in fitness, such as the development of resistance to an insecticide or a change in host-plant colonizing ability, can probably occur at least as easily in parthenogenetic populations of aphids as they can in sexually reproducing populations of many other organisms.

In the tropics, and in laboratory cultures, aphids are deprived for long periods of environmental stimuli which would normally promote sexual morph production, such as low temperature or short photoperiod. Under such circumstances it is probably that the complex physiological and chromosomal mechanisms involved in sexual morph determination and gamete production would eventually lose their ability to function due to the accumulation of mutational changes producing non-functional alleles. However it does not follow that anholocyclic life necessarily leads to an anholocyclic aphid (Hille Ris Lambers, 1966). Such mutations seem more likely to be "conditionally lethal" in their effect, resulting in the death of the clone, if or when it re-encountered sexuales-promoting stimuli. Anholocycly as an adaptive trait must presumably arise as a gene mutation (or mutations) which is actively selected for in conditions that also permit the holocycle to occur. Aphids are predominantly a temperate group, and colonization of the tropics by some cosmopolitan pest species probably occurred after the anholocyclic trait was evolved. It would be interesting to examine the response of tropical aphid populations to artificially low temperatures, to see how much latent potentiality for sexual morph production remains. Male *M. persicae* are occasionally recorded from the tropics (Blackman, 1974).

Apart from point mutations affecting the coding of particular gene products, gross genetic changes involving dissociation or rearrangement of chromosomes may occur. Again, these may be rare events in laboratory cultures, but significant when considered on the scale of field populations. Because of their holocentric structure the chromosomes of aphids are able to divide normally during mitosis even after fragmentation by X-rays (Ris 1942). Chromosome dissociations and rearrangements seem to be quite common in aphids where sufficient samples of one species have been looked at: e.g. *Myzus persicae* (Blackman & Takada, 1975) and *Amphorophora rubi* (Blackman *et al.*, 1977). Such chromosomal changes will alter the relative positions of genes and may affect their expression (see below).

Mitotic recombination

The phenomenon of mitotic recombination, whereby crossing over can occur and heterozygous marker genes can segregate in somatic cells, was first recognized in *Drosophila* (Stern, 1936). If such a process occurred in gonial cells or early cleavage nuclei of parthenogenetic aphids, it would cause progeny to differ in genotype from their parents. The use of X-rays to induce mitotic recombination in *Drosophila* has now become a major tool of developmental biology. However, spontaneous genetic recombination in somatic cells seems to be a rare event and, although it occurs in maize, yeast, and especially in fungi, it has not been conclusively demonstrated in any animal outside the Diptera (Nöthiger & Dübendorfer 1971).

Beranek & Berry (1974) suggested that mitotic recombination might account for some of the changes they observed in the enzymes of laboratory cultures of *Aphic fabae*. They calculated rates of change between 10^{-3} and 10^{-2}, too high for normal mutation and of the same order as rates usually associated with mitotic recombination. However, they did not explain how an unprogrammed genetic change such as recombination could occur simultaneously in three separate lines of *A. fabae* which had been reared in isolation from one another for seven parthenogenetic generations, as they reported for the change from 'WEBB' to 'WEBB VI'. They discussed other possible explanations, including contamination, which they maintained was "nigh impossible". However, any alternative explanation seems even less likely than the simple possibility that, despite the precautions taken, the bean seedlings used to culture the seventh generation of clone WEBB were contaminated with aphids of an alien genotype.

One may conclude that mitotic recombination is a possible course of genetic variation during aphid parthenogenesis, but is probably best regarded, if it occurs at all, as an abnormal genetic event. Like mutation, its potential significance could increase when it is considered in the context of the large size of aphid field populations.

Variability of gene expression

It has been estimated that on average only about 10% of the genes in a eukaryotic organism are being transcribed at any one time (Herskowitz, 1977: 546). Within each tissue, different genes will be active at different times, and from early development throughout life particular genes undergo complex, programmed or environmentally-conditioned changes and cycles of activity. Variability of gene expression is therefore a basic feature of life processes.

An aphid clone can in some ways be regarded as a 'superindividual' and variability of gene expression between individual aphids within a clone can occur in the same way as between tissues within an individual. This is apparent in the complex polymorphism of aphids. Some morphs (e.g. the fundatrix) are produced as part of a programmed developmental system for the clone as a whole. Other morphs are dependant on certain environmental stimuli at critical stages of the individual aphid's development, which can somehow effect a permanent change in the pattern of gene activity lasting throughout the development and adult life of that individual. It is clear, however, that such a change in the pattern of gene activity is itself ultimately under genetic control.

This is illustrated by the data for alate morph production in *M. persicae* presented above, where in spite of extreme fluctuations within clones, the genotype conditions the sensitivity to environmental factors to give a characteristic overall level of alate morph production for each clone. However, it is remarkable how rarely a complete switch to the alate morph occurred, even in clones which produced abundant alatae. Apparently the genetic switch between the apterous and late developmental pathways in *M. persicae* is sensitive enough to respond to differences in the pre- or postnatal environment between individuals born at the same time on the same leaf.

In a genetic sense, the environment can be considered at various levels: the pre- and postnatal environment of the individual, the environment of the cell within the individual, and ultimately the cytoplasmic milieu within the cell where the activity of the genes is expressed in protein synthesis. Insect epidermal hairs are unicellular structures, so in considering variability of shape, size or number of hairs one must take into account the environment at or below the level of the single cell. The genetic control of caudal hair development in *A. pisum* is apparently very sensitive at some stage to environmental factors which will ultimately affect the size and form of each hair. This caudal hair variability may be characteristic of the species as a whole (Meier, 1964), although the frequency at which altered hairs occur in any one clone presumably reflects the overall sensitivity of the control system to the environment, which is dependant on genotype.

Similar factors may decide whether a particular hair develops at all. Eastop (1973) noted that the ultimate rostral segment of *M. persicae* bears from two to seven accessory hairs in the progeny of a single parthenogenetic female, which is the variation known from the species throughout the world, whereas the subgenital plate has two hairs constantly. In contrast, the ultimate rostral segment of *Sitobion avenae* has six accessory hairs constantly, but the anterior half of the subgenital plate in this species may have two to six hairs in the progeny of a single female, which is again the range of variation known for the species as a whole. Thus it seems that variability in the expression of one feature is as characteristic of a species as is stability in the expression of another.

Spudich & Koshland (1976) demonstrated behavioural differences between bacteria grown in homogenous conditions, and suggested that the expression of certain characters within a single cell might be subject to chance. They postulated that the number of molecules of a crucial cell component, such as an mRNA, involved in the translation of certain pieces of genetic information might be small enough to be subject to Poissanian variation. They consider that non-genetic variability generated in this way could even be adaptive and aid survival of a population subjected to widely varying conditions during its lifetime.

Obviously such ideas have relevance to aphids and other apomictic organisms which go through a single cell stage between each parthenogenetic generation, as well as to structures such as epidermal hairs developing from single cells. Stochastic processes might play a prominent part, for example, in determining whether an embryo of *M. persicae* develops into an aptera or an alata under environmental conditions which do not significantly favour one or other course of development. This would remove the difficulty of having to postulate that the switching system is so extremely sensitive to minor variations in the environment. Maintenance of variability under such circumstances could also be a

considerable aid to the survival of the clone. The response to short photoperiod of clones of *M. persicae* in the intermediate reproductive category (Blackman, 1972) could also contain a stochastic element. In such clones the alatae have progeny which are intermediate between viviparae and oviparae, yet show evidence of switching of some characters to one developmental pathway or the other.

Similar considerations could apply to the non-genetic variations of hair form and number discussed above. The caudal hair alteration index of *A. pisum* appears to fluctuate randomly about a mean value and this could reflect underlying stochastic processes. There are no obvious advantages in maintaining a variability of the number of altered hairs on the cauda of *A. pisum*. However, the widespread occurrence of this variability in the species as a whole indicates that neither can there be any significance selection pressure against it.

Since the development of resistance to organophosphorus insecticides in *M. persicae,* there have been frequent reports of changes in resistance occurring within laboratory clones. Some of the examples of loss of resistance have been well-documented (e.g. Beranek, 1974a), and there is now evidence that in certain clones of *M. persicae* individual apterae can differ markedly in the amount they contain of a carboxylesterase which is closely associated with resistance (Blackman *et al.,* 1978). The intraclonal variation in resistance is apparently due to variable activity of the gene coding for the resistance-associated esterase. It now seems likely that this variable activity is associated with a particular chromosomal translocation which is found in all highly-resistant *M. persicae* clones. In *Drosophila* a chromosomal rearrangement will often give rise to a variegated or V-type position effect (Spofford, 1976). This happens when a particular gene is brought into a new location near to heterochromatin. The heterochromatin suppresses the activity of the gene in some cells but not in others, producing a mosaic or variegated effect, for example, in a character such as eye colour. Although further evidence is required, variability of resistance to organophosphorus insecticides in translocated clones of *M. persicae* could be due to a similar phenomenon.

Genetic research in recent years has revealed many other phenomena which involve changes in the activity of genetic material. Such changes may profoundly and often irreversibly alter the characteristics of the cell in which they occur, and be stable through successive mitotic divisions. In animals, it is common for whole chromosomes, and sometimes even whole chromosome sets, to be inactivated by heterochromatization. In plants, work on *Zea mays* in particular has uncovered elaborate systems of gene control mechanisms in which certain genes (controlling elements) can be transposed from one chromosomal position to another in response to signals from other genes, the changes in position of the controlling elements having a great variety of effects on the phenotype (Fincham & Sastry, 1974). One type of controlling element in maize (Spm) has been shown to undergo cyclic phase changes in its effect on neighbouring alleles, which could form the basis of a timing mechanism. Perhaps the explanation of the interval timer phenomenon in aphids (Lees, 1966) could lie in such a timing process, inherent in the genetic material itself. 'Paramutation', followed by subsequent gradual reversion to normal activity (Brink, 1973) is another possible mechanism for long term changes in gene function during aphid parthenogenesis. All these types of genetic change are classified by Serra (1968) as 'treptions', an all-embracing term which has not been widely accepted by other geneticists.

It is concluded that there are a great variety of potential ways in which variability can occur within aphid clonal lineages, involving both environment-genotype interactions and gene control processes which are inherent in the genetic material itself. The elucidation of the particular genetic mechanisms which are involved in the control of polymorphisms and other types of phenotypic variability in aphids must await a great deal of further work, which is initially dependant on the development of reliable methods of breeding aphids through their sexual phase.

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