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Genetic Polymorphism and Evolution in Parthenogenetic Animals

Part 9: Absence of Variation Within Parthenogenetic Aphid Clones*

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Summary. The enzyme gene variability within parthenogenetic clones of *Acyrtosiphon pisum* has been followed by gel electrophoresis. No variation was observed within any clone. One enzyme locus was found to vary between clones. No evidence was found to support gene recombination due to the alleged endomeiosis. This hypothesis is proven to be also theoretically untenable. The low average heterozygosity in aphids is explained as a result of directional selection operating upon the parthenogenetic aphid clones, as a consequence of which the heterozygosity is lowered.

Key words: Aphid clones – Enzyme electrophoresis – Parthenogenesis – Absence of variation

Introduction

The parthenogenetic eggs of aphids undergo a single meiotic division. This division is an equational one (White 1973; Suomalainen et al. 1976). Accordingly, the aphids have an apomictic parthenogenesis. This implies that a parthenogenetic aphid clone has no other mechanisms for genotypic change other than mutation.

De Baehr (1920) and Paspaleff (1929) observed a transient pairing of homologous chromosomes in the parthenogenetic eggs of some aphids. The chromosomes pair in young oocytes forming 'bivalents', which contract to a considerable extent. In the beginning of the growth period of the parthenogenetic oocyte, the paired chromosomes fall apart. It is generally held that there is no crossing over between homologous chromosomes and no chiasmata are formed – they would later keep the bivalents together. In contrast to this view, Cognetti (1961a, b) and Pagliai (1963, 1965) claimed to have found evidence for chiasma formation associated with this pairing, even though the bivalents would later resolve themselves into univalents. As the nuclear membrane is not dissolved, the nucleus remains diploid. It undergoes then a single mitotic division, resulting in the formation of an egg and a polar body.

A meiosis of this kind, called endomeiosis by Cognetti and Pagliai, should – according to them – make gene recombination possible. Cognetti and Pagliai used it as an explanation to account for the variability found in certain parthenogenetic aphid lines. They also relied upon this hypothesis in explaining their selection experiments on the aphid *Myzus persicae*. They report having observed a reduction in the proportion of winged forms originating from a single female until these winged forms disappeared altogether in the course of nine generations (Cognetti and Dallari 1961). Cognetti (1961b) found this evidence so compelling that he stated that 'the parthenogenesis of aphids can not be considered of ameiotic (apomictic) type'.

In order to see whether indeed there is more genetic variability within parthenogenetic aphid clones than may be accounted for by mutation, we have subjected individuals of parthenogenetic aphid clones to enzyme electrophoresis. We have chosen to study *Acyrtosiphon pisum* (Harris) (= *Macrosiphum pisi* Kalt). The senior author has elucidated the chromosome cycle of this species (Suomalainen 1933). Furthermore, Pagliai (1965) has studied the alleged endomeiosis of this species and claims that there should be variability due to endomeiotic gene recombination.

Materials and Methods

Aphid Clones

This study was made in 1978 and 1979. In each summer a single parthenogenetic *Acyrtosiphon* female was allowed to produce off-

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spring on peas planted in flower pots maintained in an isolated room. Both females were taken from a pea field aphid population in Porvoo, Finland. The field is located in a forest area, and is presumably isolated from other fields. The offspring of the females were transferred, when necessary, to fresh pea plants, which were also grown in pots. In 1978 the clone was maintained from July 16 until August 21; in 1979 the clone was started on July 13 and was maintained until October 28. This meant that about nine generations of aphids were produced within this period (the development of a single generation takes about ten days in the summer). Samples were taken from the clone at intervals of one or two weeks. In both years some parthenogenetic aphid females were taken from the same pea field as the ones previously mentioned. Lines started with these females were maintained in isolation. Only the first generation produced in culture was sampled of these clones.

Electrophoresis

The aphids were homogenized whole in a drop of distilled water. This homogenate was absorbed into filter paper strips, which were subjected to electrophoresis as described in detail by Saura et al. (1979). The following enzymes were assayed: adenylate kinase (EC 2.7.4.3); esterase (EC 3.1.1.2); fumarase (EC 4.2.1.2); glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); glycerol-3-phosphate dehydrogenase (EC 1.1.99.5); hexokinase (EC 2.7.1.1); isocitrate dehydrogenase (EC 1.1.1.42); lactate dehydrogenase (EC 1.1.1.27); leucine aminopeptidase (EC 3.4.1.1); malate dehydrogenase (EC 1.1.1.37); malic enzyme (EC 1.1.1.40); phosphoglucomutase (EC 2.7.5.1); phosphogluconate dehydrogenase (EC 1.1.1.44) and superoxide dismutase – this enzyme was observed as light areas in tetrazolium stained gels exposed to light. In addition to these assays, certain enzymes were tried without success, notably aldehyde oxidase, alcohol dehydrogenase, aldolase and triosephosphate dehydrogenase. The assay techniques are given by Saura et al. (1979). The techniques not listed in that compilation can be found in Shaw and Prasad (1970).

Controls were placed in the gels at intervals of eight aphids. Early samples of the room culture aphids were used as controls. The parthenogenetic mothers of the lines were always assayed together with their offspring.

Results

Fourteen enzymes were demonstrated successfully in aphids. Two zones of enzyme activity were found in gels stained for esterases, isocitrate dehydrogenase, leucine aminopeptidase and malate dehydrogenase. These two zones are evidently coded for by two independent loci. Accordingly, we followed eighteen loci in the aphids. A total of 216 aphids were studied in 1978. Of these, 76 were the offspring of the female taken into room culture in July 16. A variable number (from 14 to 30) of aphids were studied of the first generation progeny of six females, which were isolated separately. In 1979 we sampled the offspring of the female taken into isolation in July 13 at intervals of one or two weeks. The size of the samples was variable, ranging from 5 (the last surviving adult aphids of October 28) to 34 individuals. A total of 204 aphids was assayed in 1979.

Eggs, which indicate the emergence of the amphigonic (sexual) generation, were found beginning in mid-October in 1979. The last two samples of October contained both males and amphigonic females. They should theoretically be identical with their parthenogenetic mothers.

No variation was observed within any clone studied. The samples of two consecutive years were also identical with each other. The only variability observed within this study was within clones. A single enzyme was involved, namely malate dehydrogenase-1. This is the form of malate dehydrogenase, which stays at the point of insertion of samples in the gel. The other form migrates some cm towards the anode. Two of the clones taken into isolation separately in 1978 had three bands of malate dehydrogenase-1, suggesting that the aphids of these clones were heterozygous and possessed two allozymes of this enzyme. One allozyme was identical to that found as the only malate dehydrogenase-1 in all other aphids, while the other migrated about 10 mm towards the anode. This enzyme type was also found in the mothers of these clones (numbers 4 and 6). One enzyme phenotype, that of glycerol-3-phosphate dehydrogenase, consisted of three bands in the aphids. This phenotype could be mistaken as representing the product of two different alleles. Heterozygotes normally have three bands of this dimeric enzyme. All aphids studied by us had, however, clearly a single form of this enzyme. The explanation is that the insect glycerol-3-phosphate dehydrogenase is composed of three bands, which appear to be of about equal strength in aphids (Bewley et al. 1974).

Discussion

Numerous electrophoretic studies have been made on the between clone variation in aphids (May and Holbrook 1978; Baker 1979). In comparison with this, little has been done to elucidate variation within a clone. Wool et al. (1978) noted that there were no differences between individuals of the same clone. Likewise, May and Holbrook (1978) found that heterozygosity was preserved within a clone and state that the parthenogenesis of aphids is apomictic. Both of these within clone studies were made on *Myzus persicae*. In contrast to these studies Beranek and Berry (1974) and Beranek (1974) report that they have found within clone variability at esterase loci in *Aphis fabae* and *Myzus persicae*. They explain this variability as a result of gene recombination due to the alleged endomeiosis.

In this study we have followed an aphid clone for over three months. Within this period at least nine generations of aphids emerged. Each one of the more than 200 aphids

of this clone studied was identical for eighteen enzyme loci. This result, as well as the absence of variability within any of the other clones of *Acyrtosiphon pisum* studied by us indicate clearly that aphids have an apomictic parthenogenesis. Gene recombination may occur only at the meiosis of the sexual generation and at the fertilization following meiosis.

Authors, who have reported finding evidence for genetic change within parthenogenetic aphid clones, in general attribute their results to gene recombination associated with the alleged endomeiosis (Beranek and Berry 1974 and in particular Beranek 1974 and references therein). The opinion that the alleged endomeiosis makes within clone gene recombination possible in aphids, has been expressed recently in certain major treatises on the subject. Even though a transient pairing of the homologous chromosomes occurs in the parthenogenetic eggs of aphids, the senior author concluded some time ago (Suomalainen 1950) that the chromosomes do not cross over and that no chiasmata are formed.

Blackman (1978) has studied the oogenesis of parthenogenetic aphids in detail. He presents ample evidence to support his view that the hypothesis of endomeiosis is untenable and should be discarded. We may add that even if the eggs of parthenogenetic aphids should undergo a crossing over as suggested by Cognetti (1961a, b), this would not result in gene recombination. The nuclear membrane of the parthenogenetic oocyte is not dissolved in the course of 'endomeiosis'. Following the transient pairing all chromosomes remain in the same nucleus, which then undergoes a mitotic division (the sole maturation division). All genes remain within the same nucleus. Accordingly, the hypothesis put forward by Cognetti and his school, that the 'endomeiosis' mechanism described by them would result in gene recombination, is also theoretically untenable. (That is, the only possible effect endomeiosis might have is associated with the *cis-trans* effect).

Beranek and Berry (1974) report rates of genetic change within a parthenogenetic aphid clone. They are 2.3×10^{-3} and 6.25×10^{-3}. These values are, of course, far too high to represent mutation rates. We have sampled here about 14 000 enzyme genes without observing a single variant. If we assume that the mutation rates for enzyme genes are, on average, of the same order of magnitude, the differences between our results and those of Beranek and Berry become highly significant.

Beranek and Berry (1974) and Beranek (1974) report that clones started with a single female produced variable progeny in controlled conditions. The variant types bred true, so that the changes should have been genetically controlled. Only one of the variants described by Beranek in *Aphis fabae* could be explained as the result of a heterozygote having reverted to a homozygote, while the other types can not be accounted for by recombination. The rates of occurrence of these new types do not suggest mutation as a cause. Our results indicate that the detectable mutation rates for aphid enzyme genes are not higher than those for any other genes. 'Endomeiosis' evidently does not exist. Even if it did exist, it would not – as we have shown – explain the results of Beranek and Berry.

It is a common observation that there is very little between clone variation in several aphid species (Wool et al. 1978; May and Holbrook 1978; Baker 1979). The level of average heterozygosity in aphids is low compared with insects in general (Powell 1975). We noted one variable enzyme, malate dehydrogenase-1, in this study. There is a single exception to the low average heterozygosity, namely *Macrosiphum euphorbiae*. It has an average heterozygosity of 0.07, which is rather low for an insect but within the range of variation of insects in general (Powell 1975). The authors of this observation (May and Holbrook 1978) attribute the low heterozygosity in *Myzus persicae* to founder effect in its American populations, while *Macrosiphum euphorbiae* represents, in their opinion a native American species with high heterozygosity. We find this interpretation improbable, as there has certainly been enough time and aphids to allow for mutation to operate since the introduction of American *Myzus persicae* from, say, China. Baker (1979) has found a comparably low level of heterozygosity for *Myzus persicae* in Scotland. This undermines even further the founder effect explanation for low heterozygosity.

We present here an alternative explanation. In the spring, the variable offspring of the sexual generation of *Acyrtosiphon pisum* commence parthenogenetic reproduction. There is probably competition between different genotypes, with the result that the fittest ones prevail later in the summer. The number of clones may be high in the spring, but this variability is reduced in the course of the parthenogenetic generations through between clone competition. Reproductive capacity is probably not a limiting factor for parthenogenetic aphids. Consequently, a majority of autumn individuals within a field may represent the offspring of a few sexual females. We may note that the parthenogenetic clones studied by us had an identical overall enzyme phenotype over all loci. The situation is comparable to our observation of little variability in the flying beetle *Bromius (Adoxus) obscurus* (Lokki et al. 1976). The sexual generation in aphids represents, of course, the offspring of the parthenogenetic generations. The allele frequencies of the sexual generation represent the end result of selection, which has operated upon the parthenogenetic generations. Selection has evidently been mostly directional in *Acyrtosiphon pisum* and *Myzus persicae*. In apomictic parthenogenesis the effect of selection is opposed only by mutation. In cyclical parthenogenesis the phase of transient polymorphism may be very short (that is, the period during which a favorable allele replaces

a less favorable one with concomitant genetic substitution load). In cyclical parthenogenesis a beneficial mutant may become common in the parthenogenetic generations. It has then a very high probability of becoming fixed in the next sexual phase. This is our explanation for the low level of enzyme gene heterozygosity in *Acyrtosiphon pisum*.

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