Genetic Variation, Clonal Selection and Balance Polymorphism in Natural Populations of Cyclic Parthenogenetic Rose Aphids (Macrosiphum rosae).

By

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ABSTRACT

This study was undertaken to assess the role of natural selection in the maintenance of genetic variation in natural population of Rose Aphids (Macrosiphum rosae). The selection of rose aphids as organism of choice was based on three features common to all aphids. (1) Their habitat with respect to food (host-plants) is well defined, (2) Their migration potential is large enough that geographical differentiation of allele frequencies are not expected, and (3) Aphids are cyclic parthenogenetic which allows to investigate the role of seasonal change and variation in breeding system on the amount and pattern of genetic variation. A total of nine geographic (Detroit, London, Hamilton, Niagara Falls, Rochester, Syracuse and New York City) and eleven local populations (Hamilton, Ontario) were studied for one morphological and fourteen allozyme loci. On an average about thirty percent of loci were polymorphic and an individual was heterozygous for about 4.3% of its genome. All geographical populations except Detroit and New York had roughly similar allele frequencies. The latter two populations differed somewhat from the rest and these changes are thought to be due to temporal rather

than geographical factors. The local variations at eleven sites in Hamilton was remarkably similar to the macrogeographic pattern of variation. Six local populations in Hamilton were studied for temporal variation using four polymorphic loci. All loci showed large genotypic fluctuations over time but the variation at only one locus (Esterase-4) was cyclic in nature. However, the occurrence of intermediate allele frequencies and the temporal changes at all four polymorphic loci strongly suggest that these polymorphism are maintained by balancing selection. The balancing selection in this case is thought to be of the type where genotypes have different fitness during the sexual and asexual generations. Since during the asexual generations, selection acts in the whole genome (clonal selection) any directional change in allele frequencies, whether due to natural selection or hitch hike, must be balanced presumably by changes during the sexual generation. In this view the cyclic parthenogenetic breeding system appears to be directly involved in shaping the structure of genetic variation in rose aphids.

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CHAPTER 1 INTRODUCTION

The discussions about the nature of genetic variation and its significance for adaptive evolution have to some extent always been coloured by the methods employed to study genetic variation. Before the introduction of molecular methods to population genetics, the problem of estimating the amount of genetic variation and showing its significance for adaptive evolution were essentially one and the same problem, i.e., to show that genetic variation was of adaptive significance, as proposed in the balance hypothesis (Dobzhansky, 1955), was to show that plenty of genetic variation existed in natural populations of various organisms. This was so, because under the alternative hypothesis, the classical hypothesis, one simply did not expect the existence of such variation.

Then it would seem paradoxical that after plenty of enzyme and protein variation has been shown to exist in natural populations of almost any organism which has been studied (Powell, 1975; Nevo, 1978; Selander, 1976) its significance for adaptive evolution still remains a matter of controversy (Lewontin, 1974). This is not to say that the individual workers themselves are undecided about the significance of allozyme variation - quite the contrary. With few exceptions (e.g., see Mukai and Veolker, 1977) most experimental population geneticists seem to implicate the role of natural selection in the maintenance of allozyme polymorphism

although with varying degrees of certainty. On the other hand, the classical hypothesis, or what is now called the neutral hypothesis, proposes that most allozyme variation is neutral and is maintained by balance between mutation and random genetic drift (Kimura, 1968; Nei, 1975; Fuerst et al., 1977). A new variation of this hypothesis is that allozyme variants are slightly deleterious and maintained by balance between mutation and selection (Ohta, 1974). Thus after fifteen years of intensive study of this problem the general concensus appears to be that the adaptive significance of allozyme variation in population is, at the best, uncertain. Thus while to some (mostly selectionists), both natural selection and random genetic drift may seem to play a role in the maintenance of protein polymorphism and the problem is to decide how much of a role does each factor play, to others (mostly neutralists) the main problem still remains to decide if protein polymorphism have any role to play at all in adaptive evolution.

The problem seems to reside in the fact that many of the observations on allozyme polymorphism presented as evidence in favour of the selection hypothesis can also be explained by the netural hypothesis. Some of the features of allozyme variation that have been presented as evidence in favour of the selection hypothesis are: Geographical uniformity of allele frequencies (Lewontin, 1974; Ayala et al., 1972), geographical and altitudinal clines (Clegg and Allard, 1972; Koehn and Rasmussen, 1967; O'Gower and

Nicol, 1968; Grossman et al., 1969), correlation between heterozygosity and the environment (Powell, 1971; Ayala and McDonald, 1974; Hedrick, Ginevan and Ewing, 1976), seasonal variation in gene and genotype frequencies (Hebert, 1974; Smith and Fraser, 1976; Young, 1979; Steiner, 1979) and linkage disequilibrium (Prakash and Lewontin, 1968; 1971; Charlesworth and Charlesworth, 1979; Langley et al., 1979, 1977; Mukai et al., 1974). In terms of the classical hypothesis, the geographical uniformity of allele frequency can be explained by assuming some migration between populations (Kimura and Maruyama, 1971; Kimura and Ohta, 1971), geographical clines like that reported by Hamrick and Allard (1972) in Avena barbata can be due to migration, and correlation between heterozygosity and environment can be fortuitous (Anderson et al., 1975). Linkage disequilibrium between loci can result from non selective forces such as population mixing, population bottleneck and hitch-hike (Hedrick et al., 1978). On the other hand, observations on seasonal variation and altitudinal clines can provide unequivocal evidence in favour of natural selection but such observations are relatively rare (Steiner, 1979).

A second problem has been that in various studies of geographical variation while the organisms

differ in their breeding systems and population-structures, rather similar kind of data have been gathered in all these studies. Only recently, attempts have been made to make use of the variation in breeding systems and population structures in deciding the issue of selection vs. neutrality (Hebert, 1974a,b; Lokki et al., 1976; Jaenike et al., 1980). The rationale would be to choose an organism that because of its breeding system, population structure and life-cycle, can provide an unequivocal evidence in favour of the selection hypothesis. Aphids are such an organism and they are the subject of this study.

Aphids have three features which make them a very suitable organism for studying genetic variation. (1) They breed by cyclic parthenogenesis, i.e. each year they have a sexual generation followed by several asexual generations consisting of females only. Since any selective difference among genotypes produced in the sexual generation would be magnified during the asexual generations, aphids provide an excellent opportunity for measuring fitness differences between genotypes. Also, since different aphid species have different degrees of sexuality, they would be excellent material to evaluate the effect of sexuality vs. asexuality on genetic variation. (2) Aphids are well

known for their mass migration and consequently the entire species can be regarded as a single breeding unit. This means that we should not expect any geographic differentiation between populations and if such variation did exist it would be a strong evidence in favour of the selection hypothesis. (3) Some aphid species use different host-plants during the sexual and asexual generations. Also, while some aphid species prefer a wide variety of secondary hosts and are, consequently, cosmopolitan in nature, others have a much more restricted host range. Thus the variation in their host specificity would make aphids an excellent material for testing the niche-variation hypothesis.

This study was undertaken with Rose aphids (Macrosiphum rosae L.) to study: (1) the geographic patterns of genetic variation at both micro and macro levels, (2) seasonal variation in gene and genotypic frequencies which may be due to migration or natural selection, and (3) development of linkage association between polymorphic loci which may be expected due to the cyclic sexual-asexual breeding system employed by this species. The pattern of seasonal variation in morph colour and allozyme frequencies shows that natural selection is an important factor in shaping the pattern of genetic variation observed in this species.

CHAPTER 2 MATERIALS AND METHODS

(i) Life Cycle: Rose aphids (Macrosiphum rosae) are monophagous and feed on roses and seem to prefer wild roses to cultivated ones. Exploitation of the host-plant resource is achieved by a complex system of morphs, each form having a different function. When the plant is actively growing in spring, the predominent form in the temperate region is a wingless, parthenogenetic and viviparous female (aptera) which are produced from overwintering eggs. Rapid embryonic and nymphal development result in a rapid increase of their population size. The nymphs produced by these parthenogenetic females include a variable proportion of individuals which develop wings in the adult stage (alate). These are also parthenogenetic viviparous female, but their progeny are characteristically wingless. Thus the winged forms are well adapted to disperse the species and help them colonize other host-plants. The migration which occurs as a result of high population density and deteriorating food resource permits the dispersal of the species over a wide range.

This mode of reproduction goes on until late October or beginning of November when sexual forms,

triggered by low temperature and/or short photoperiod are produced (Kenten, 1956; Lees, 1959; Blackman, 1975). Rose aphids have five pairs of chromosomes and sex determination is of xx and xo type, xo being male. Winter is passed in the form of overwintering fertilized eggs which produce parthenogenetic females of the next generation (Fig. 1). In the tropics, on the other hand, sexual forms do not seem to be produced at all and the aphid reproduces by parthenogenetic means all the year round (Maelzer, 1977).

(ii) Sample Collection: Aphids were collected from rose bushes growing in private or public gardens from nine geographical areas (Fig. 2). These localities are: Detroit (Michigan), London (Ontario), Delhi (Ontario), Troy (Ontario), Hamilton (Ontario), Niagara Falls (Ontario), Rochester (New York), Syracuse (New York) and New York City (New York). The Hamilton area was sampled in detail to provide a microgeographic picture of variation. A total of eleven local sites (numbered from H1 - H11) were sampled (Fig. 2). For seasonal variation, six of these sites (H1, H4, H7, H8, H9 and H11) were sampled more than once at an interval of about two months between late May and early November. All samples, except those collected at the beginning of winter (late October - early November), consisted of parthenogenetic females only.

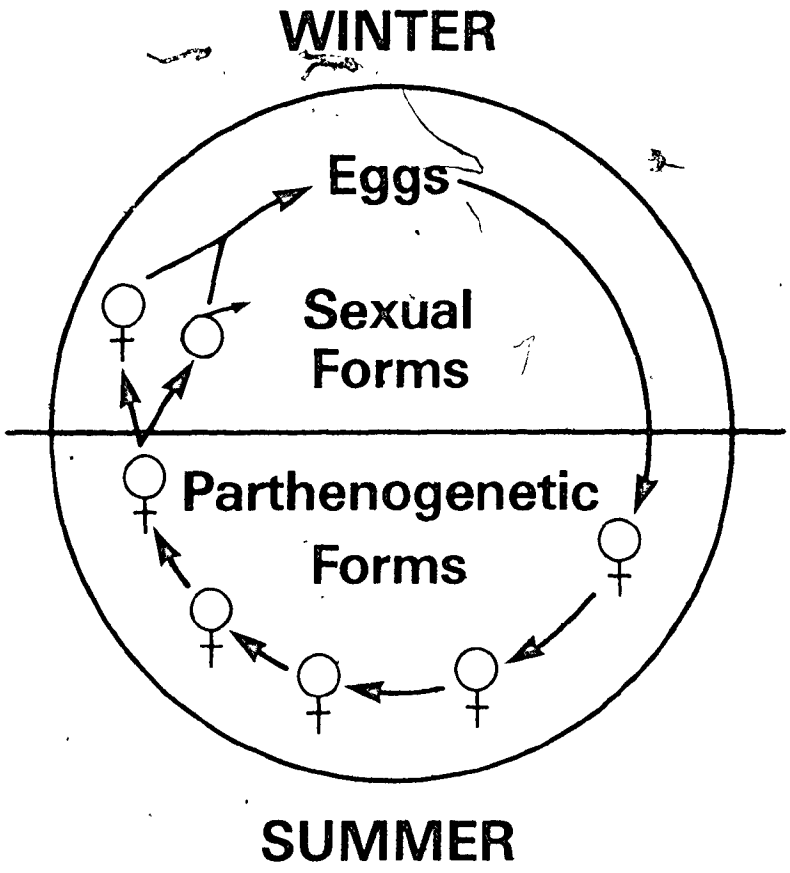

Figure 1. Life Cycle of Macrosiphum rosae.

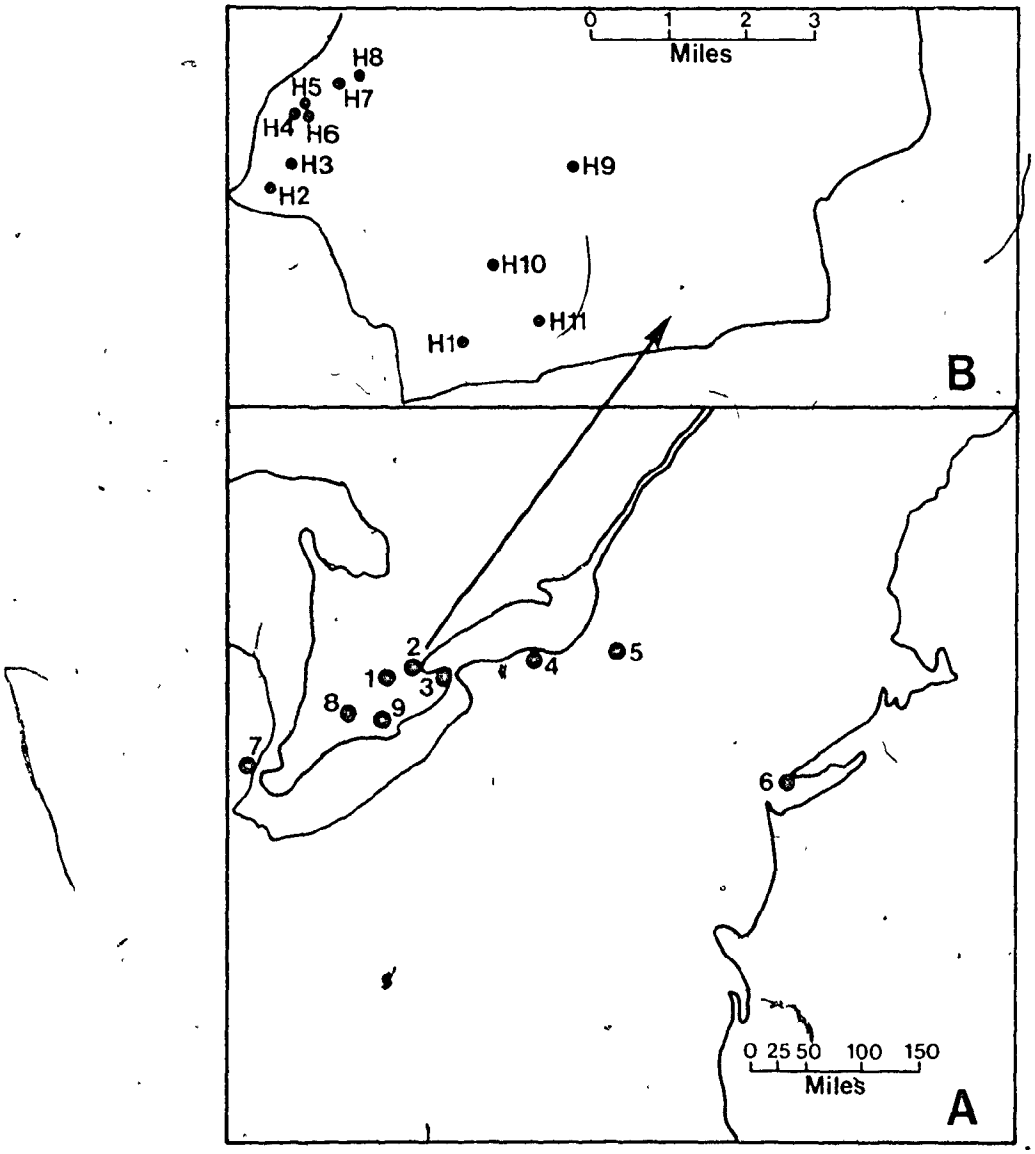

Figure 2. Geographical locations of populations studied.

(A) 1 = Troy (Ontario), 2 = Hamilton (Ontario), 3 = Niagara Falls (Ontario), 4 = Rochester (N.Y.), 5 = Syracuse (N.Y.), 6 = New York (N.Y.), 7 = Detroit (Michigan), 8 = London (Ontario), 9 = Delhi (Ontario).

(B) Microgeographic locations of various sites in Hamilton (Ontario).

Aphids were gently tapped from rose bushes and brushed into the bottle. Only mature individuals were collected. They were brought to the lab alive and were examined morphologically to remove any contamination from other species. Occasionally I found individuals of a very distinct phenotype with large abdomen and which showed extra allozyme bands for some enzyme systems. These individuals are thought to be infected with parasites (Wool, Van Embden and Bunting, 1978) and were discarded. The aphids were scored for their morph colour (red vs. green) and then were either used immediately for gel electrophoresis or frozen at $-60^{\circ}F$ for later use.

(iii) Electrophoretic Technique: Electrophoresis was carried out on polyacrylamide slab gels using standard methods described by Prakash, Lewontin and Hubby (1969). The gel boxes used were from Aardvark Instruments, Inc. (Chicago). Various buffer systems and staining methods were tried and modified to give the best results (Shaw and Prasad, 1970; Harris and Hopkinson, 1975). A total of ten enzymes coded by fourteen loci were examined (Table 1).

Table 1. A list of enzyme loci and buffers used for Electrophoresis.

	Enzymes	Number of loci	Gel and Electrophoresis buffer		Buffer source*
1.	Acid phosphatase (ACPH)	1	.1M Tris Borate EDTA	pH 8.9	A
2.	Esterase (EST)	4	.1M Tris Glycine	pH 8.5	B
3.	Glutamate dehydrogenase (GDH)	1	.1M Tris Borate EDTA	pH 8.0	C
4.	Glutamate oxaloacetate transaminase (GOT)	1	.1M Tris Borate EDTA	pH 8.0	C
5.	Leucine amino peptidase (LAP)	1	.1M Tris Borate EDTA	pH 8.0	C
6.	Malate dehydrogenase (MDH)	1	.1M Tris Borate	pH 8.2	D
7.	Malic enzyme (ME)	1	.1M Tris Borate	pH 8.2	D
8.	Octanol dehydrogenase (ODH)	1	.1M Tris Borate EDTA	pH 8.9	A
9.	Sorbitol dehydrogenase (SDH)	1	.1M Tris Glycine	pH 8.5	B
10.	Tetrozolium oxidase (TO)	2	.1M Tris Borate EDTA	pH 8.0	C

* Buffer source: A = Prakash, Lewontin and Hubby (1969)
B = Morton and Singh (1980)
C and D = Shaw and Prasad (1970)

CHAPTER 3 RESULTS

In my initial screening I did not detect any activity in single aphid homogenates for several enzymes. This problem has also been reported in other aphid species (Wool et al., 1978a; Tomiuk and Wohrmann, 1980). Thus, out of a total of twenty-four enzymes initially screened, only ten showed good enough activity to be scored reliably (Table 1). These ten enzymes are coded by a total of fourteen loci of which four are polymorphic. These are Esterase-2 (Est-2), Esterase-4 (Est-4), Sorbital dehydrogenase (Sdh) and Malate dehydrogenase (Mdh). The three polymorphic allozyme loci (Est-2, Est-4 and Sdh) and a red/green morph colour locus were used for studying seasonal and geographical variation; Mdh was dropped because it did not stain consistently well enough to be scored reliably in all samples.

Both Est-2 and Est-4 had two alleles each and both showed a two-banded heterozygote which suggests these enzymes are monomers. Sorbitol dehyrogenase had two alleles and showed a five-banded heterozygote which suggests that the enzyme is a tetramer. At the red/green morph colour locus only two types, red and green, were observed and it is not obvious as to which type, if any, is the dominant form. To show that the red and green morphs were not due to the colour of the plant foliage the aphids had been feeding, a green house experiment was done in which the red types were

kept on green (mature) foliage and the green types were kept on red (young) foliage for several generations. As it was already obvious from our observations on natural populations, the colour of the foliage has no effect on the colour of these morph types. The only change in colour I have observed is in the red types at the approach of winter when the temperature begins to drop. The red types sometimes change to yellowish-orange colour which is probably due to physiological changes in the host plant and/or the organism. The four polymorphic loci (Est-2, Est-4, Sdh and morph colour) were used to study the pattern of variation and the results are presented as follows:

(i) Microgeographic Variation:

The frequency of the red and green morph types in eleven local populations in Hamilton are presented in Table 2. All local populations are quite polymorphic and with exception to one site (H-11) there is a tendency for the green morph to be more common in all populations.

The data for Est-2, Est-4 and Sdh locus are presented in Table 3, 4 and 5 respectively. There is a complete lack of heterozygotes at Esterase-2 locus. The frequency of both homozygotes, SS and FF, are rather similar in all populations. At Est-4 locus there is a deficiency of one homozygote type (FF) in all populations except in H-11 where both homozygotes (SS and FF) are lacking in frequency. Only three of these sites were studied for Sdh locus. All three populations are in Hardy-Weinberg equilibrium but the

Table 2. Frequencies of red and green morph types at various sites in Hamilton population of Macrosiphum rosae.

Sites	Date	Morph Types		N	No. of host plants
		Red	Green		
H-1	9 July'78	.25	.75	197	4
H-2	26 June'78	.44	.56	82	3
H-3	28 June'78	.49	.51	168	15
H-4	8 June'78	.34	.66	332	35
H-5	22 June'78	.39	.61	403	10
H-6	22 June'78	.37	.63	172	15
H-7	22 June'78	.48	.52	494	7
H-8	22 June'78	.47	.53	343	4
H-9	2 June '78	.44	.56	206	8
H-10	28 June'78	.16	.84	61	10
H-11	1 June'78	.80	.20	243	6
	mean	.42	.58		
		.38	.62	(excluding H-11)	

Table 3. Genotypic frequencies at Esterase-2 locus at various sites in Hamilton population of Macrosiphum rosae.

Site	Genotype SS	SF	FF	X^2	N
H-1	.74	-	.26	45.88***	47
H-2	1.00	-	-	-	43
H-3	.87	-	.13	46.10***	46
H-4	.94	-	.06	50.00***	50
H-5	.87	-	.13	46.10***	46
H-6	.78	-	.22	45.15***	46
H-7	.80	-	.20	45.00***	45
H-8	.98	-	.02	49.79***	45
H-9	.98	-	.02	50.08***	52
H-10	.80	-	.20	44.52***	46
H-11	1.00	-	-	-	36

Significance level: * = $p < .05$, ** = $p < .01$, *** = $P < .005$

Table 4. Genotypic frequencies at Esterase-4 locus at various sites in Hamilton population of Macrosiphum rosae.

Site	Genotype			X^2	N
	SS	SF	FF		
H-1	.68	.32	-	1.68	47
H-2	.26	.74	-	15.16***	43
H-3	.74	.26	-	1.04	46
H-4	.66	.32	.02	0.35	50
H-5	.74	.26	-	1.04	46
H-6	.80	.20	-	.82	46
H-7	.58	.42	-	3.24	45
H-8	.91	.09	-	0.16	45
H-9	.50	.50	-	5.78**	52
H-10	.87	.13	-	0.31	46
H-11	.03	.97	-	32.26***	36

Significance Level: * = $p < .05$,
** = $p < .01$,
*** = $p < .005$

Table 5. Genotypic frequencies at Sorbitol dehydrogenase locus at various sites in Hamilton populations of M. rosae.

Site	Genotype			X^2	N
	SS	SF	FF		
H-4	.67	.33	-	1.82	45
H-7	.71	.25	.04	0.73	45
H-9	.35	.52	.13	0.42	46

genotypic frequencies at site H-9 are different from those at sites H-4 and H-7.

Thus, with exception to site H-11, the local sites in Hamilton population show rather similar pattern of variation which is what we would expect from the nearness of these sites and the migration potential of this species.

(ii) Macrogeographic Variation:

The frequency of the red and green morph types in nine geographical populations, including Hamilton, is given in Table 6. The figures for Hamilton are the weighted mean of the eleven sites studied there. As it was the case in local populations of Hamilton, all geographical populations have high frequency of the green morph type. The discrepancy between the frequencies of two morph types is less pronounced in Hamilton and London populations. Note that the sample sizes from these two populations are larger than others. Syracuse and New York populations show the lowest frequency of red types (6% in both) and as a group appear different from all other populations.

The genotypic frequencies for Est-2 and Est-4 are given in Table 7. For Est-2 only Hamilton, Niagara Falls and Rochester show common polymorphism, others are monomorphic or nearly so. Again, like Hamilton population, no heterozygotes was detected in any of the polymorphic populations. All populations are polymorphic for Est-4 but there is a complete absence of one homozygote (FF) in all populations. Among the polymorphic populations, Syracuse and New York are similar at Est-2 locus

Table 6. Proportions of red and green morph types in geographical populations of *M. rosae*.

Populations	Date	Morph Types		N	No. of host-plants
		Red	Green		
Detroit	6 Aug '79	.19	.81	75	6
London	6 Aug '79	.47	.53	208	5
Delhi	26 Aug '79	-	1.00	160	5
Troy	17 July '78	.27	.73	66	7
Hamilton	10 June '78	.42	.58	2701	107
Niagara Falls	18 July '78	.28	.72	141	50
Rochester	4 July '78	.21	.78	67	10
Syracuse	4 July '78	.06	.94	130	21
New York	4 July '78	.06	.94	70	12
Mean		.22	.78	3618	

Table 7. Genotypic frequencies and test of Hardy-Weinberg equilibrium at the Est-2 Est-4 locus in various populations of M. rosae.

Populations	Esterase-2				Esterase-4				N
	SS	SF	FF	X^2	SS	SF	FF	X^2	
Detroit	1.00	-	-	-	.91	.09	-	0.21	69
London	1.00	-	-	-	.94	.06	-	1.14	69
Delhi	1.00	-	-	-	.41	.59	-	20.62***	98
Troy	1.00	-	-	-	.55	.45	-	10.09***	66
Hamilton	.89	-	.11	498.84***	.61	.39	+	59.35***	502
Niagara Falls	.84	-	.16	68.46***	.78	.22	-	3.82	69
Rochester	.71	-	.29	77.96***	.71	.29	-	5.45*	65
Syracuse	.98	-	.02	97.27***	.90	.10	-	2.10	93
New York	.99	-	.01	99.38***	.96	.06	-	1.15	69

Significance level = * = $p < .05$, ** = $p < .01$, *** = $p < .005$

but with respect to Est-4 locus, they are also similar to Detroit and London populations. The other populations (Delhi, Troy, Hamilton and Rochester but not Niagara Falls) have significant deficiency of fast homozygote at Est-4 locus.

The genotypic frequencies at Sdh locus are presented in Table 8. Three populations (London, Delhi and Niagara Falls) shared significant deviation from Hardy-Weinberg proportions but while London and Niagara Falls populations were deficient in the frequency of heterozygotes, Delhi had an excess of heterozygotes. With exception to Troy where both SS and SF genotypes were equally common, and Delhi where SF and FF genotypes were equally common, in all other populations the SS homozygotes was the most common form. Again New York and Detroit were similar but quite close to being monomorphic.

Thus with respect to genic variation all population had the same two alleles at each polymorphic loci, but most importantly, eight out of nine populations showed a significant deviation from Hardy-Weinburg equilibrium for one or more loci. Also with one exception (Sdh locus in Delhi population) the deficiencies in all populations were for the same genotype.

(iii) Seasonal Variation:

Six local populations in Hamilton were sampled several times over a period of six months, from June to November in 1978. To compare the genotypic frequencies before and after winter, these populations were again sampled in June 1979, just after the new aphids had appeared. Furthermore

Table 8. Genotypic frequencies at Sdh locus in geographical populations of M. rosae.

Populations	Genotypes			X^2	N
	SS	SF	FF		
Detroit	.94	.06	-	0.06	69
London	.80	.13	.07	15.49***	69
Delhi	-	.48	.52	9.49***	98
Troy	.48	.52	-	2.87	23
Hamilton	.57	.37	.06	1.01	136
Niagara Falls	.78	.09	.13	10.54***	23
Rochester	.87	.13	-	0.15	23
Syracuse	.65	.35	-	1.95	46
New York	.96	.04	-	0.02	23
Mean	.67	.24	.09		

to see if the patterns of changes were cyclic, two populations (H-4 and H-9) were also sampled in August and October of 1979. The patterns of changes in genotypic frequencies at the morph colour, Est-2 and Est-4 loci are shown in Figs. 3, 4 and 5 respectively. Dramatic changes have occurred in the frequency of red morph over a period of six months. While in some populations the changes seem to be cyclic (e.g. in H-1, H-4, H-9), in others (e.g. H-7, H-8, H-11) the changes are directional. Thus among six populations there is no overall similarity in the pattern of change, suggesting that the morph colour locus alone is not the site of natural selection producing these changes.

Similarly for Est-2 locus H-1, H-4 and H-9 sites showed significant changes as compared to H-7, H-8 and H-11, but no cyclic pattern was apparent at this locus.

Contrary to the morph colour and Est-2 locus, changes observed at Est-4 locus are cyclic and similar in four of the six populations studied (Fig. 5). In all popualtions the frequency of heterozygote (SF) decreased during summer and was lowest at the end of the fall season (October-November). After winter, however, the frequency in most populations has increased dramatically producing a cyclic pattern of change. The seasonal pattern of change at Est-4 locus is shown more clearly in Fig. 6 where the data from 1978 and 1979 are pooled together

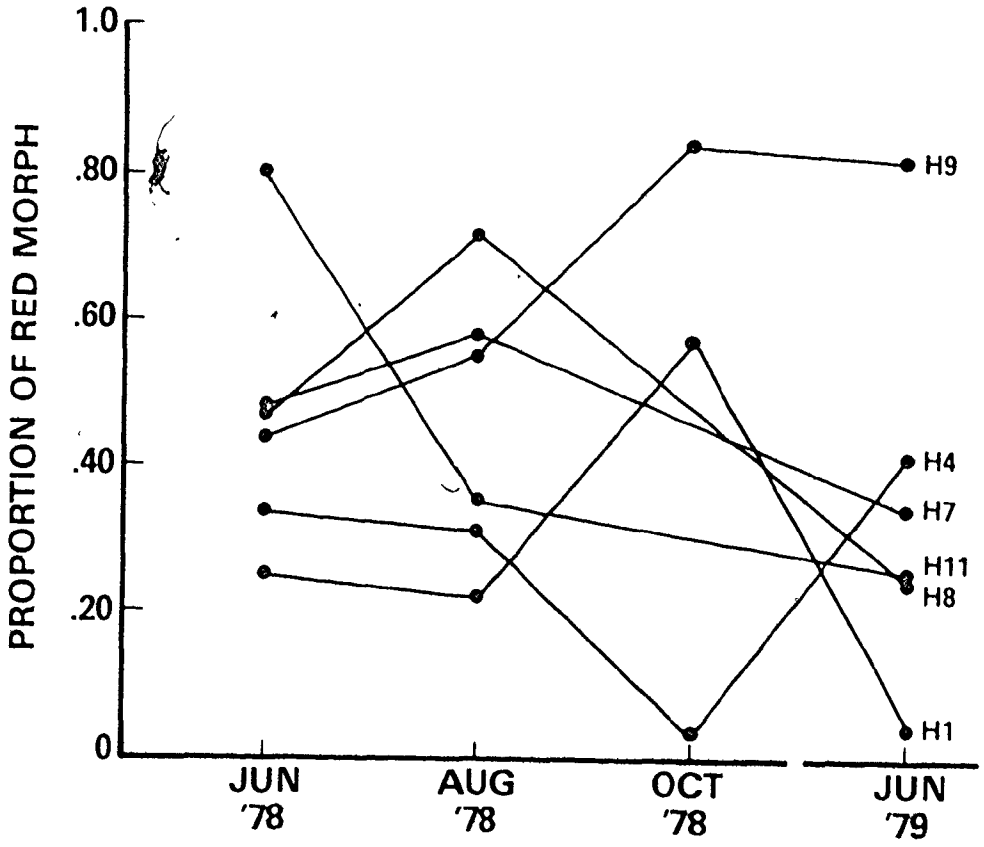

Figure 3. Temporal changes in the frequency of red morph type at various sites in Hamilton populations.

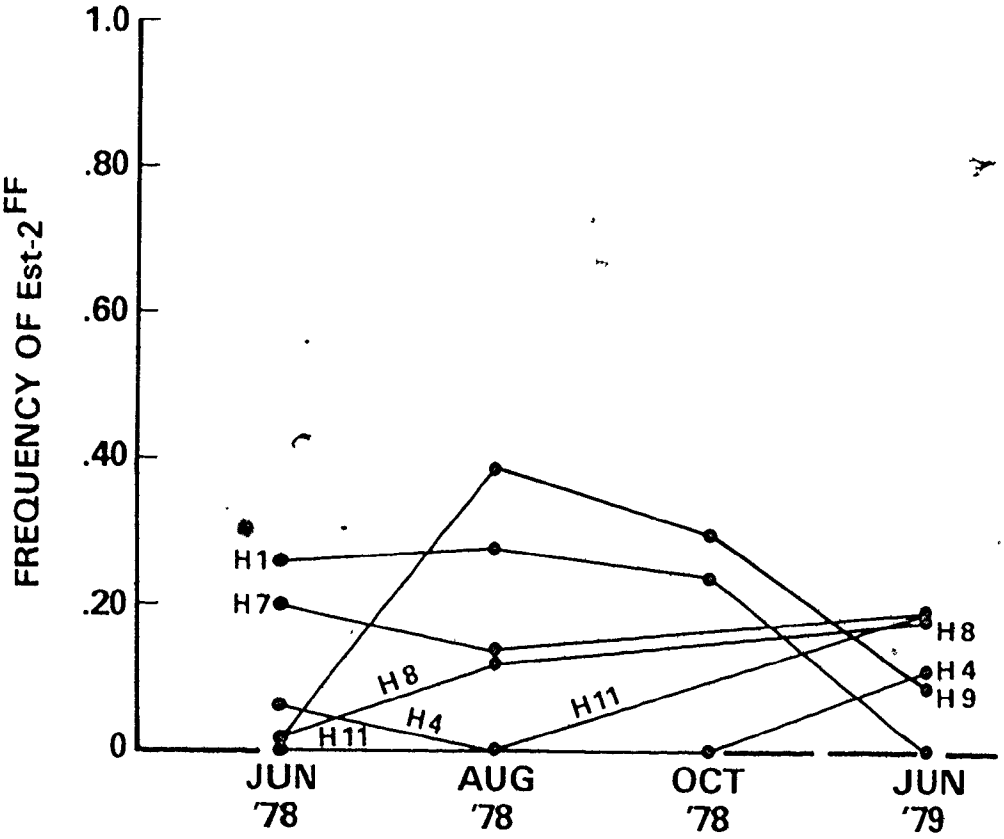

Figure 4. Temporal changes in the frequency of FF genotype at Esterase-2 locus at various sites in Hamilton populations.

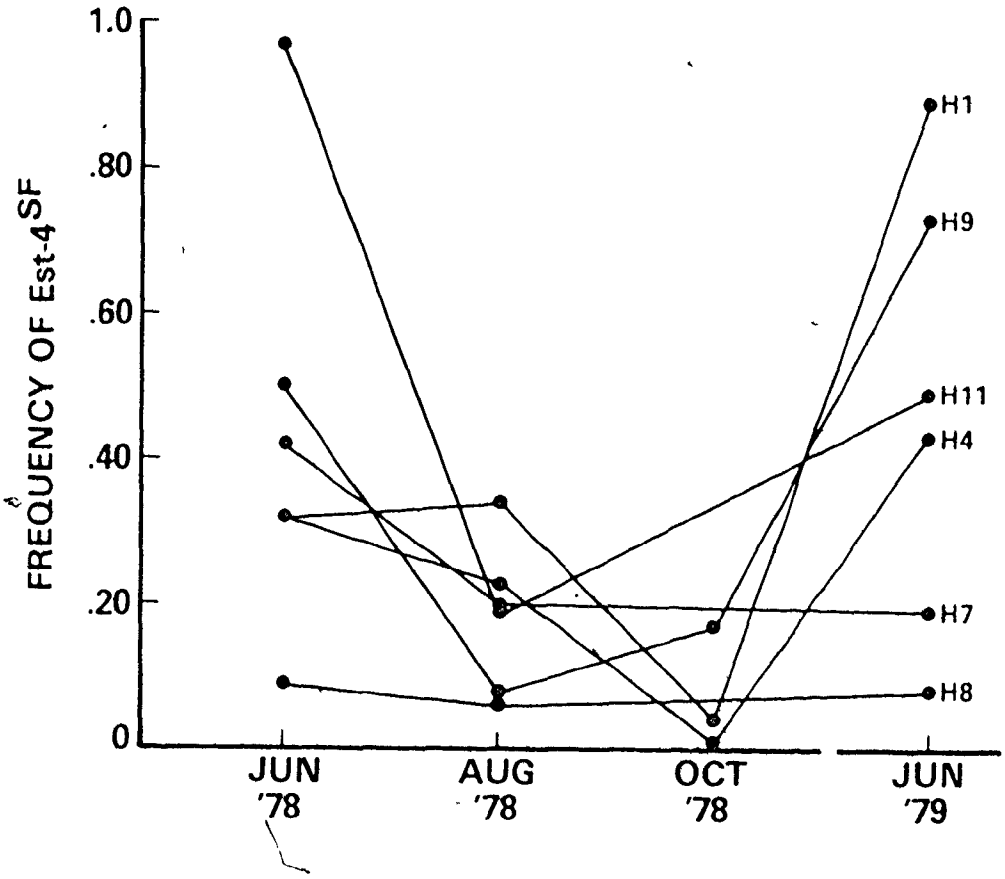

Figure 5. Temporal changes in the frequency of SF genotype at Esterase-4 locus at various sites in Hamilton population.

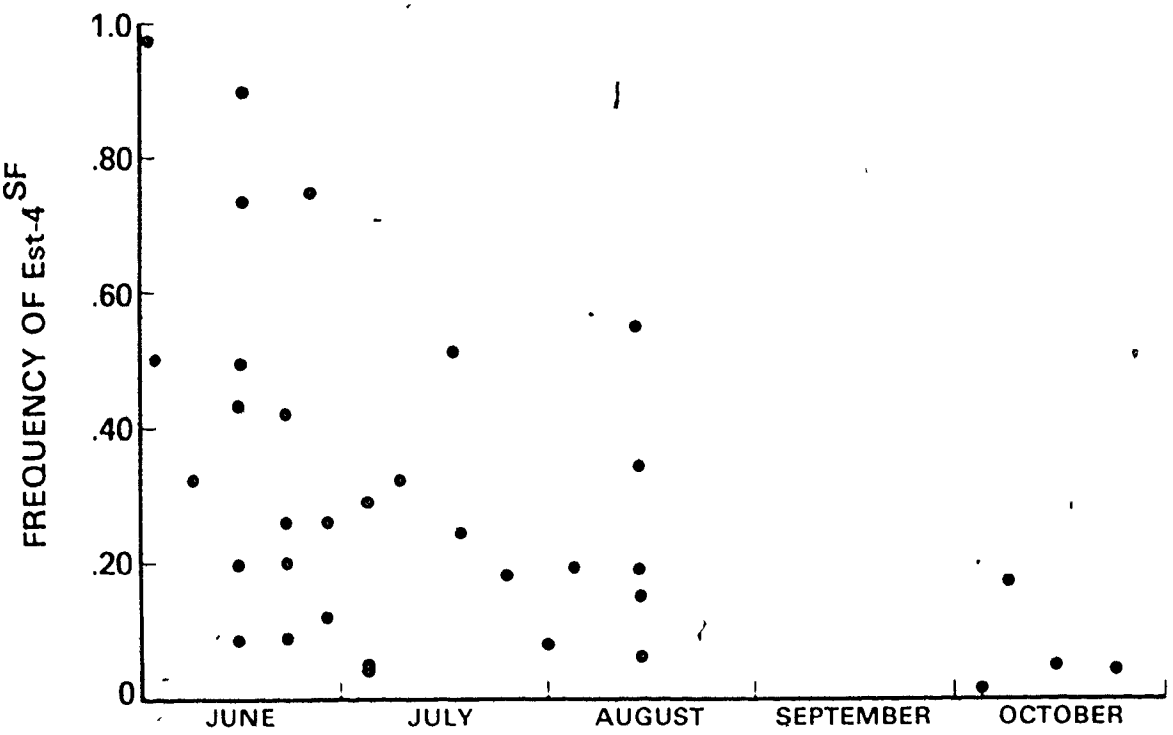

Figure 6. Overall temporal changes in the frequency of SF genotype at Esterase-4 locus in all geographic populations. Combined data from 1978 and 1979.

It is clear that the frequency of heterozygote is highest in spring when new aphids appear and there on it decreases, being lowest at the end of fall season. There is a general deficiency of FF genotype and it appears that the selection is also against the heterozygote at this locus. It is tempting to suggest that these changes are correlated with temperature but we have no direct data to show the causal relationship. Changes of such magnitude (up to 60%) over a six months period are truly remarkable and must involve extremely powerful selection. The system appears to resemble the behaviour of a lethal gene but if so, then one is left to wonder as to why such genes have not been altogether eliminated from the natural populations. While the seasonal changes at Est-2 and morph colour loci were not consistent in all populations, to see if there was any overall increase or decrease in the frequency of any one type, frequencies from all populations were plotted against the date of sampling (Figs. 7 and 8). While at Est-2 locus there is still no seasonal pattern, the morph-colour locus shows an overall increase in the frequency of the red type which must be counter balanced during the sexual generation. Thus the variation at the morph colour locus also experiences a cyclic change but it is not as dramatic as that at the Est-4 locus.

The changes in genotypic frequencies at Sdh locus in three local Hamilton populations are presented in Table 9.

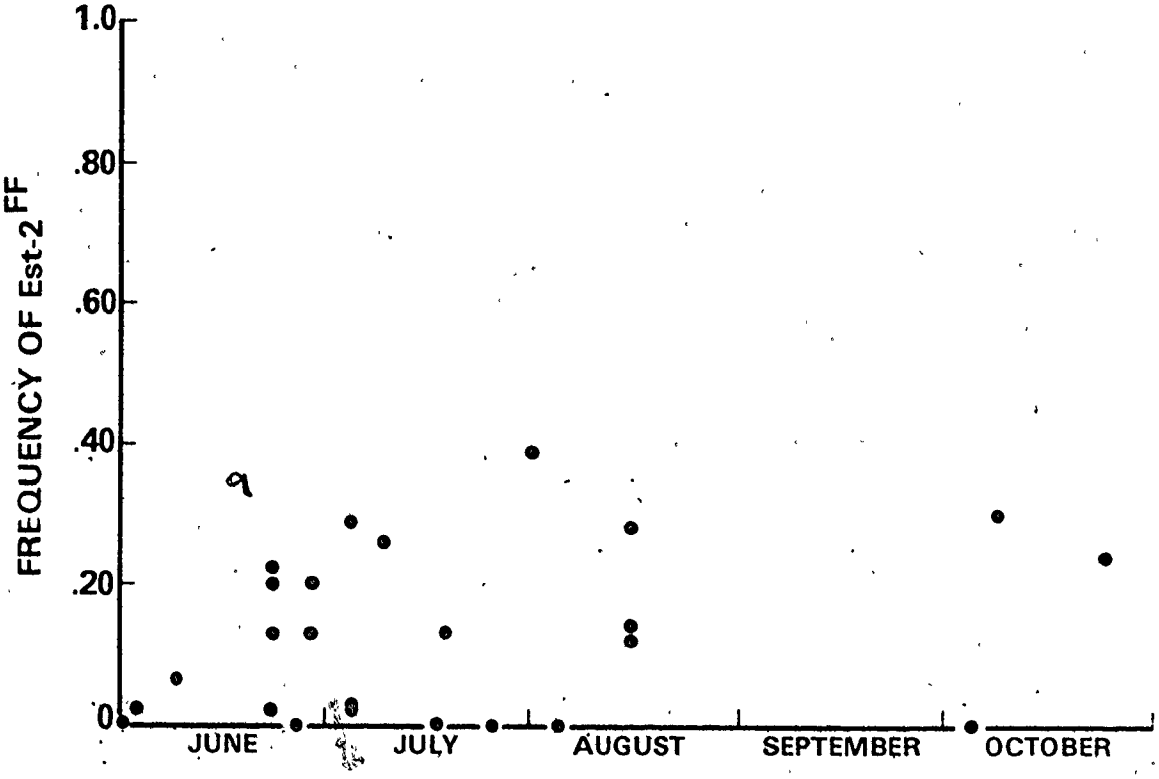

Figure 7. Overall temporal changes in the frequency of FF genotype at Est-2 locus. Combined data from 1978 and 1979.

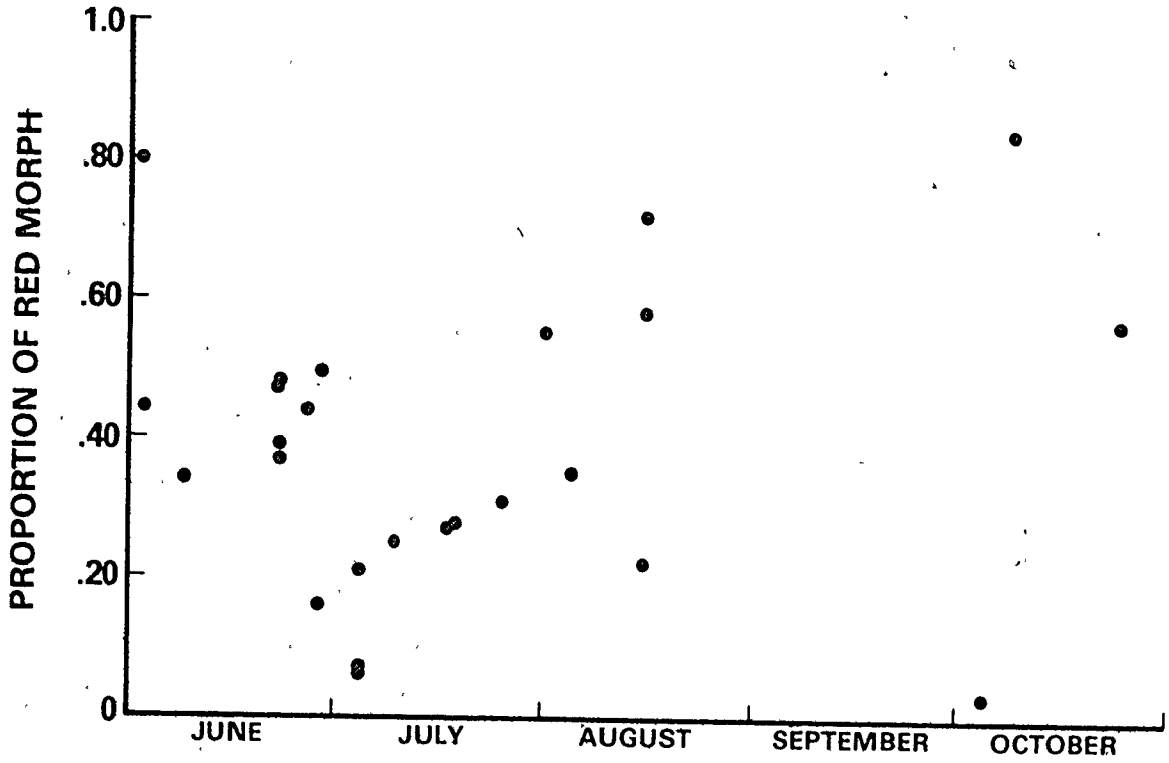

Figure 8. Overall temporal changes in the frequency of red type in all geographic populations. Combined data from 1978 and 1979.

Table 9. Seasonal changes in genotypic frequencies and test of deviation from Hardy-Weinberg equilibrium at Sorbitol dehydrogenase locus in geographical populations of Macrosiphum rosae.

Population Site	Genotype	1978 June	1978 August	1978 October	1979 June	1979 August	1979 October
Hamilton (H-4)	SS	.67		1.00	.68	.54	.61
	SF	.33		-	.14	.39	.34
	FF	-		-	.18	.07	.05
	N	45		46	57	46	92
	X^2	1.75		-	21.50***	.04	.08
Hamilton (H-7)	SS	.71	.46		.40		
	SF	.25	.54		.55		
	FF	.04	-		.05		
	N	45	46		53		
	X^2	.62	6.45*		3.11		
Hamilton (H-9)	SS	.35	.40	.50	.26	.53	
	SF	.52	.36	.50	.70	.43	
	FF	.13	.24	-	.04	.04	
	N	46	45	22	46	23	
	X^2	.41	3.31	2.43	9.76***	.35	
Delhi	SS				-	-	
	SF				.48	.72	
	FF				.52	.28	
	N				98	46	
	X^2				9.73***	14.35***	

Significance level: * = $p < .05$; ** = $p < .01$; *** = $p < .005$

All three populations show significant within and between-year changes but the changes are not consistent among populations, i.e. the same genotype shows a temporal increase in one population but a decrease in another. In contrast to these populations where the FF homozygote is either absent or rare, in Delhi it is the SS homozygote which was altogether absent and FF homozygote was the most common genotype in June 1979. It was suspected that this might be due to random genetic drift, the population was reexamined in August 1979 and it showed a significant decrease in the frequency of SF. These changes are consistant with our hypothesis that on the average the F allele has a lower fitness. However, since changes in the genotypic frequencies are not in the same direction in all populations, these changes are thought to be due to linked gene(s) which is marked by the Sdh locus.

Since aphids respond to density and/or physiological deterioration of hosts by producing winged individual who disperse and colonize new hosts, I thought may be the seasonal changes are occurring due to genotypic differences in the dispersal rates of various genotypes. Even though all six local populations showed the same trend at Esterase-4 locus which makes this possibility very unlikely, I nevertheless tried to test it directly. Both winged and non-winged females were examined

for their morph colour and allozyme variation and the result of the test of independence between pairs of loci characteris is shown in Table 10. Significant associations are rare which means that changes in genotypic frequencies are not due to differential migration of different genotypes. Beside, if the seasonal changes were due to differential migration, then changes at all loci should be in the same direction as in aphids the migration is of the whole genome and not of individual genes. This of course did not occur which leaves me to propose that the seasonal changes are caused by natural selection. Significant association also did not occur between the morph colour and allozyme loci. Since all polymorphic loci did show seasonal changes, this lack of association between polymorphic loci would call for a very high rate of total natural selection occurring in this species.

(iv) Clonal Selection:

Since selection during asexual cycle is on the whole genome rather than on single genes, I decided to take a look at the overall pattern of change with respect to all polymorphic loci. For this, individuals were classified into clonal types on the basis of their genotypes at Est-2, Est-4 and the red/green morph colour locus. Sdh locus was not included as all four loci could not be scored in single aphids. The frequencies of various clones are presented in Table 11 for the local population in Hamilton, and in Table 12 for the geographic populations.

Table 10. A list of significant and the total number of pairwise comparisons made for association among Est-2, Est-4 and the morph type loci.

Character Pair	Number of Comparisons	Significant at 5% level	Population with Significant Association
Red/green - Est-2	16	0	-
Red/green - Est-4	16	2	Troy, H2
Est-2 - Est-4	16	2	New York and Syracuse
Winged/non-winged - Est-2	16	1	New York
Winged/non-winged - Est-4	16	2	H2,H9
Winged/non-winged - Red/green	16	0	-

Table 11. Frequency of various clone types and test of random association of genotypes at 3 loci in six local populations of Macrosiphum rosae.

Clone Type	Genotype MC	E-2	E-4	H-1	H-4	H-7	H-8	H-9	H-11
A	Red	SS	SS	.19	.25	.20	.26	.17	-
B	Red	SS	SF	-	.08	.17	.04	.33	.94
C	Red	FF	SS	-	.03	.07	-	.02	-
D	Red	FF	SF	-	-	.02	-	-	-
E	Green	SS	SS	.28	.35	.26	.63	.31	.03
F	Green	SS	SF	.30	.25	.17	.04	.17	.03
G	Green	SS	FF	-	.02	-	-	-	-
H	Green	FF	SS	.21	-	.07	.03	-	-
I	Green	FF	SF	.02	.02	.04	-	-	-
			N	47	50	45	45	52	36
			X^2	15.46*	6.64	.77	5.40	7.70	8.13*

Table 12. Frequency of various clone types and test of random association of genotypes at 3 loci (morph colour, Est-2 and Est-4) in various populations of Macrosiphum rosae.

Clone Type	Detroit	London	Delhi	Troy	Hamilton	Niagara Falls	Rochester	Syracuse	New York
A	.10	.44	-	.20	.18	.07	.07	.05	-
B	.07	.01	-	.07	.15	.05	-	-	-
C	-	-	-	-	.01	.03	.06	.01	-
D	-	-	-	-	.01	-	-	-	-
E	.82	.50	.44	.33	.37	.60	.46	.83	.95
F	.01	.05	.56	.36	.20	.15	.26	.10	.05
G	-	-	-	-	+	-	-	-	-
H	-	-	-	-	.06	.10	.16	.01	.01
I	-	-	-	.04	.02	-	.05	-	-
N	69	69	98	66	502	69	65	93	69
x^2	14.73***	1.82	.35	3.44	25.82***	10.13	9.93	1.48	6.91

Since Est-2 is weakly polymorphic, most individuals fall into four major colonal types (A, B, E and F). The same four major types are common locally as well as geographically. However, in contrast to the general similarity between populations on the basis of single genes, there are significant differences among some populations in the frequency of these clones, i.e., the populations become much more differentiated from each other when the genotypes based in all polymorphic loci are taken into account.

Since seasonal changes in genotypic frequencies were observed at all polymorphic loci but the changes at only Est-4 locus were consistent in all populations, I would expect that the extent of seasonal changes in clonal types would be significant but would not be consistent, i.e., would not be in the same direction in all populations. This appears to be the case from the data presented in Fig. 9 for the four clonal types on the basis of the two highly polymorphic loci, Esterase-4 and morph colour. These two loci had shown significant seasonal changes and the exclusion of Est-2 has no overall effect on the pattern seen in Figs. 9a,b,c. The frequency changes are of large magnitudes and sometimes cyclic but often in the opposite direction in different populations. I think this is because three polymorphic loci obviously do not represent the genome and the changes seen here are probably due to a very large number of loci segregating in these populations.

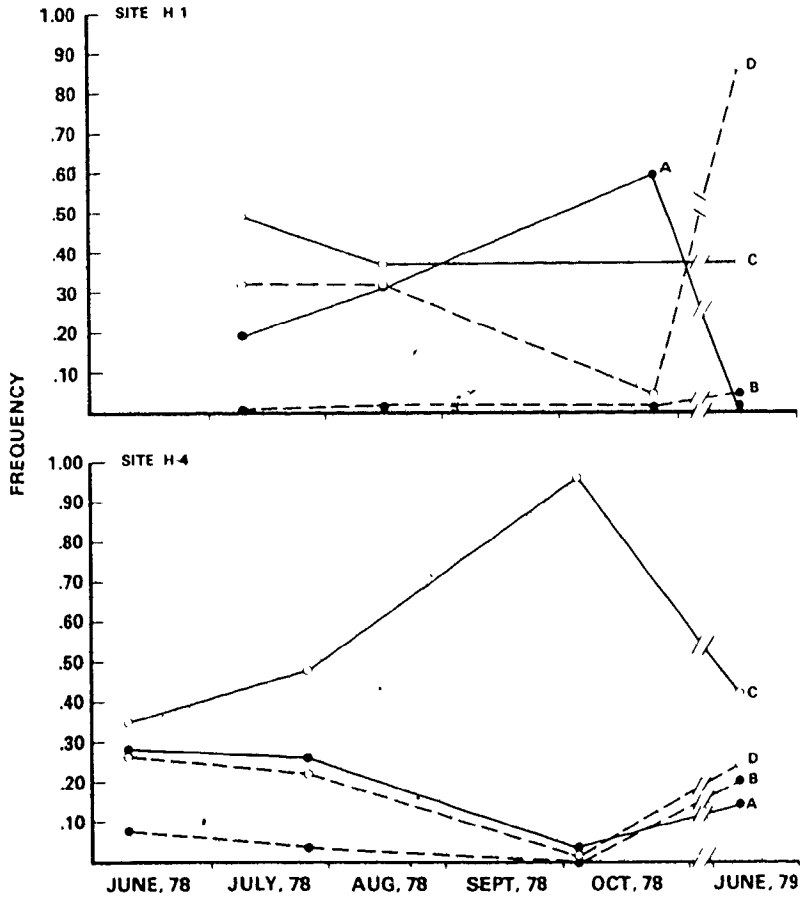

Figure 9a. Cyclic changes in frequencies of clonal types at site H-1 and H-4 in Hamilton (Ontario). Clonal types based on the morph colour and Est-4 are: A = Red, SS
B = Red, SF
C = Green, SS
D = Green, SF

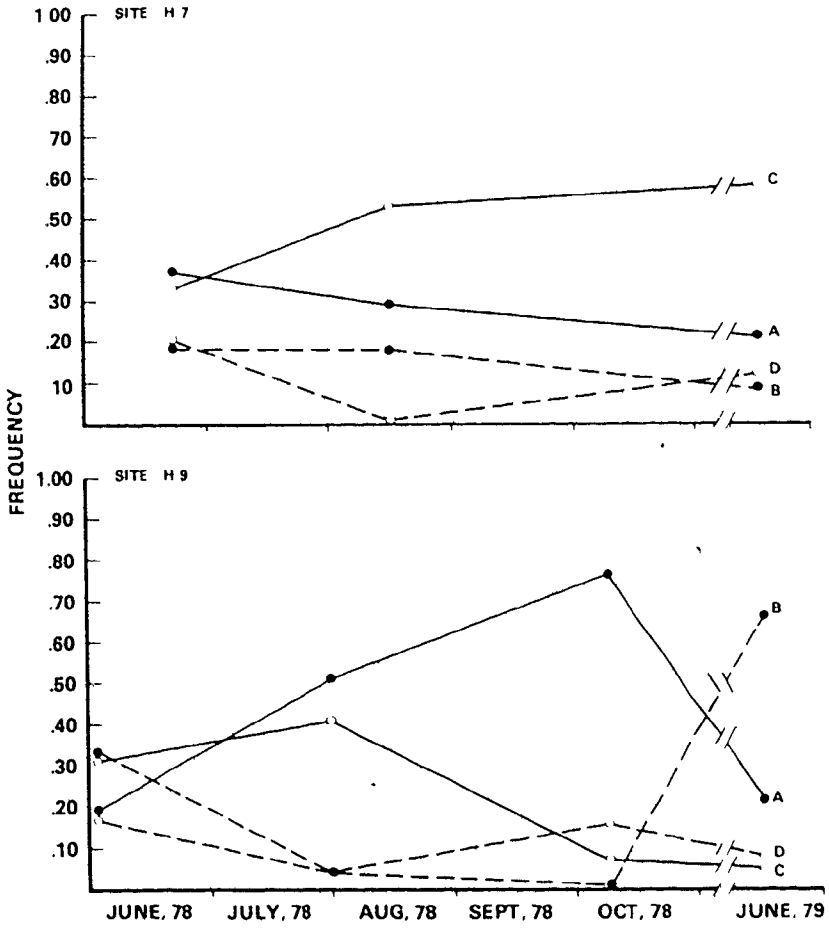

Figure 9b. Cyclic changes in frequencies of clone types at site H-7 and H-9 in Hamilton (Ontario). For clone types see legend for Figure 9a.

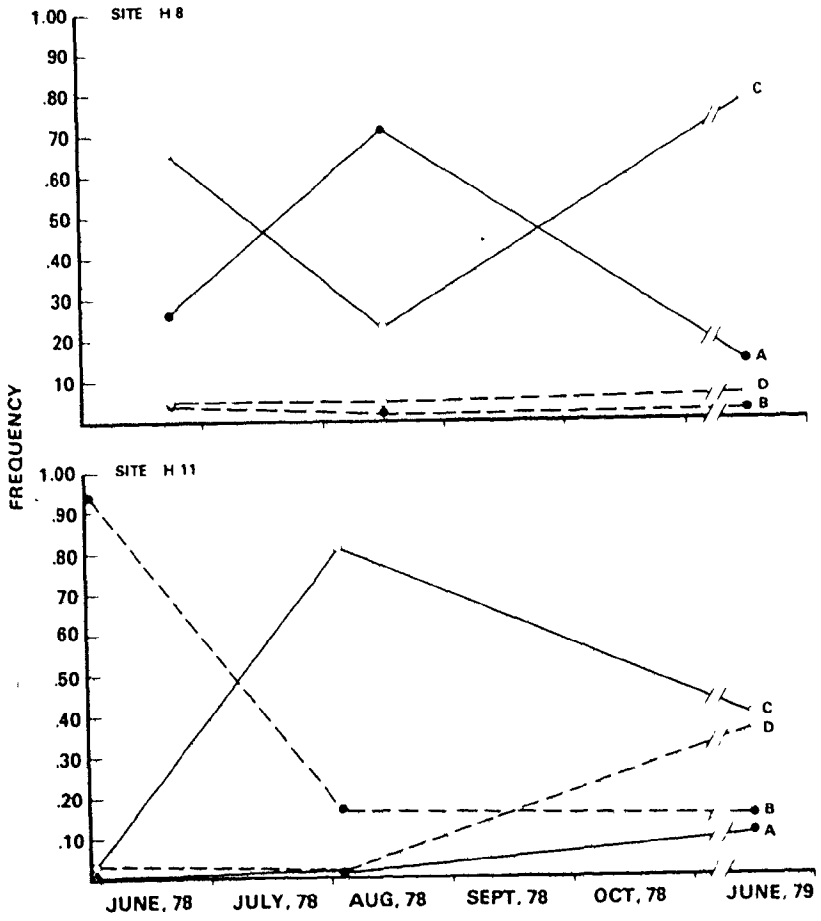

Figure 9c. Cyclic changes in frequencies of clone types at site H-8 and H-11 in Hamilton (Ontario). For clone types see legend of Figure 9a.

CHAPTER 4 DISCUSSION

Due to cyclic parthenogenetic breeding system and its association with seasonal changes, the results from Macrosiphum rosae have important bearing on at least three aspects of genetic variation in general. These are: (1) geographic pattern of variation, (2) seasonal changes in variation, and (3) clonal selection. In the following each of these aspects will be discussed separately as they relate to the findings of this study.

(i) Geographical Pattern of Variation:

The genetic variation in nine geographic populations of M. rosae is summarized in Table 13. On the average 30% of loci are polymorphic, and based on the 13 allozyme loci, the average heterozygosity is 4.3%. Tomiuk and Wohrmann (1980) studied twenty loci in German population of M. rosae and found 10% loci to be polymorphic and an average heterozygosity of 4.4%. If we combine the two studies the proportion of polymorphic loci becomes 20% and the average heterozygosity 4.35%. Compared to many Drosophila species (Lewontin, 1974; Powell, 1975; Nevo, 1978) these figures are low, the average heterozygosity being much lower than the proportion of polymorphic loci. However, compared to many species of aphids, M. rosae seems to be rather much more variable. A number of studies on various species of aphids are summarized

Table 13. Proportion of polymorphic loci and average heterozygosity in geographical populations of Macrosiphum rosae.

Population	No. of polymorphic loci [1]	Proportion of polymorphic loci	Average Heterozygosity[3]	
			Polymorphic loci (3)[2]	all loci (13)
Detroit	4	.266	.048	.011
London	4	.266	.097	.022
Delhi	3	.200	.259	.059
Troy	4	.266	.244	.056
Hamilton	5	.333	.323	.074
Niagara Falls	5	.333	.250	.058
Rochester	5	.333	.259	.059
Syracuse	5	.333	.177	.041
New York	5	.333	.029	.006
Mean	4.44	.295	.187	.043

1. Including Mdh and morph colour loci
2. Based on only three allozyme loci (Est-2, Est-3 and Sdh)
3. Excluding Mdh and morph colour loci

in Table 14. In many studies only a few variable loci were employed for the study and hence they do not provide a general picture of the genetic variation in these species. However there are several studies where a sufficient number of loci have been studied to make a valid comparison. May and Holbrook (1978) studied 18 loci in two populations of Macrosiphum euphorbae and 19 loci in nine populations of Myzus persicae. They showed that M. euphorbae has 28% of its loci polymorphic and an average heterozygosity of about 7%. Contrary to this, Myzus persicae was found to have very little variation. Tomiuk and Wohrmann (1980) studied nine species of aphids and found that genetic variation was quite low. But there are some differences in the level of variation in the same species in different continents. For example, A. pisum appears to be more polymorphic in Germany than in Finland and M. euphorbae is more variable in USA than in Germany. Similarly M. persicae shows more variation in English populations than in American populations. It is not clear if these differences between geographically distant populations are real or simply the result of technical differences in various laboratories or due to sampling of different loci in different studies. Clearly more studies need to be done on similar sets of loci in different populations of the same species. It is interesting to note that of the aphid species studied in detail, the two species which do show variation (M. euphorbae and M. rosae) belong to the same genus. It would be interesting to survey more members of both Myzus and Macrosiphum genera and see if

Table 14. Geographic locations of populations, number of loci studied, proportion of loci polymorphic and average heterozygosity in various species of Aphids.

Species	No. of Populations or strains and location [1]	No. loci sampled [2]	% loci polymorphic	Av. Het.	Source
Acyrtosiphon pisum	-, Germany	7	.43	-	Tomiuk and Wohrmann (1980)
	420S, Finland	18	.05	-	Suomalainen et al. (1980)
Aphis fabae	25S, England	(Alkaline phosphatase)			Furk (1979)
	2, England	(2 Esterase loci)			Beranek and Berry (1974)
	5, England	(2 Esterase and Acid phosphatase)			Beranek (1974)
	-, Germany	25	.08	-	Tomiuk and Wohrmann (1980)
Aphis pomi	-, Germany	12	0	0	Tomiuk and Wohrmann (1980)
Aphis sambuci	-, Germany	9	.11	.037	Tomiuk and Wohrmann (1980)
Macrosiphum euphorbae	2, U.S.A.	18	.28	.07	May and Holbrook (1978)
	-, Germany	8	0	0	Tomiuk and Wohrmann (1980)
Macrosiphum funestum	-, Germany	7	0	0	Tomiuk and Wohrmann (1980)
Macrosiphum rosae	-, Germany	20	.10	.044	Tomiuk and Wohrmann (1980)
	9, U.S.A. and Canada	14	.29	.043	This study
Myzus persicae	1, England	(6 esterases			Sudderuddin (1973)
	3, Scotland	(3 esterases, Acid phosphatases)			Baker (1978, 1979)
	30, England	(3 esterases, Acid phosphatases)			Beranek (1974)
	3, Japan	(5 Esterases			Takada (1979)
	35 S, England	11	.18	-	Wool et al. (1978)
	9, U.S.A.	19	0	0	May and Holbrook (1978)
	1, Canada	7	0	0	Joseph and Singh (unpublished

...2

Table 14 cont'd.

Species	No. of Populations or strains and location [1]	No. loci sampled [2]	% loci polymorphic	Av. Het.	Source
Rhodobium porosum	-, Germany	13	0	0	Tomiuk and Wohrmann(1980)
Wahlgreniella nervata	-, Germany	10	.20	.037	Tomiuk and Wohrmann(1980)

1. - means no. of populations sampled is not clearly stated; S stands for strains.

2. In studies where only frequently polymorphic esterases and phosphatases have been examined, the number of loci studied are given in bracket but detail data are not available.

this difference is a general one.

With respect to heterozygosity, the nine populations of M. rosae seems to fall into rather two discrete groups. Delhi, Troy, Hamilton, Niagara Falls, Rochester and Syracuse are quite similar and make one group, and Detroit, London and New York, another. Thus there is a suggestion in the data to show that the southern populations are less variable than the northern ones. Later I will show that this difference is due to seasonal changes in the genetic variation. The rarity of some genotypes in southern populations may be due to the fact that these genotypes are eliminated during asexual generation, a process which starts in the southern populations earlier than in northern populations. The similarity of geographic populations, except the variation due to seasonal changes, is expected in aphids due to the fact that migration is quite common in these organisms. Thus, this study suggests that in those species where migration is seasonal (as is the case in aphids), single sampling of geographical populations is not sufficient to provide a true picture of genetic variation in natural populations.

The pattern of genetic variation in Macrosiphum rosae appears to be very similar to Daphnia which also utilizes a cyclic parthenogenetic breeding system (Hebert, 1974a,b). The extent and the organization of genetic variation in cyclic parthenogenetic organisms seems to fall in between sexual and

completely asexual species (Suomalainen et al., 1980; Nur, 1977; Lokki et al., 1976, Jaenike et al., 1980; Stille et al., 1980; Mitter et al., 1979; Angus, 1980; Vrijenhoek, 1978; Schneider, 1978; Scudday, 1973). Generally in asexual organism, (1) fewer loci are polymorphic, (2) polymorphic loci show strong deviations from Hardy-Weinberg proportions, (3) genotypes at various loci are strongly associated and (4) species with strong migration potential have less genetic variation.

The study of genetic variation in aphid species can also shed some light on the niche-variation hypothesis. The niche-variation hypothesis, predicts that species with wide niche should show more genetic variation than the species with narrow niche (Van Valen, 1965). Inspite of the fact that there is considerable difficulty as to how to measure the niche-variation of a species, a number of attempts, of both ecological as well as genetic nature, have been made to test the association between genetic variation and niche width with mixed results. Van Valen (1965) hypothesized that variation in bill size in birds would be correlated with the variety of food and habitats used by a population. However, Soule and Stewart (1970) found no such correlation between variation of bill characters and the variety of foods taken by six species of Central African birds from Malawi and Zambia and suggested that the niche-variation hypothesis may not hold for 'complex' characters. Shugart and Blaylock (1973) showed that increase of variation (through

mutagenesis) was found to increase the niche width of highly inbred populations of D. simulans. Prakash showed that D. buskii has very little genetic variation which was supposedly due to its narrow niche (narrow geographic distribution). Jaenike and Selander (1979) studied genetic variation in Drosophila falleni, a fungus feeding species, and found very little genetic variation inspite of its ability to feed on a variety of mushroom species. Sabath (1974) examined the relationship between genetic variation and niche-width in eleven sympatric species of Drosophilidae and found no correlation. On the other hand, Steiner (1977, 1980) has shown a positive relationship between heterozygosity and the number of plants used for oviposition sites in several species of Hawaiian Drosophila. In aphids, of the three well studied species, Myzus persicae, Macrosiphum euphorbae and Macrosiphum rosae, M. persicae has very little variation even though it is polyphagous and utilizes a variety of secondary host plants. On the other hand, both M. euphorbae and M. rosae are more variable than M. persicae, but while M. euphorbae is polyphagous and utilizes a number of secondary host plants, M. rosae is monophagous and to my knowledge occurs only on roses. Thus there does not appear to be a strong correlation between the genetic variation and the niche-width of a species. There could be a variety of reasons why this is so, and I will mention only a few. Jaenike and Selander (1979) concluded that inspite of the fact that D. falleni feeds on a variety of mushroom species these mushrooms

are unstable environment as far as D. falleni is concerned and because of this there has been a selection for a general purpose genotype. Another explanation would be that the strength of association between genetic variation and niche-width of a species would be a function of two opposite forces: selective differences between different genotypes to exploit different kinds of niches (e.g. different host-plants in aphids) and the ability of a single genotype to utilize a variety of niches. If the host specific differences between different genotypes of a species are small or only as large as the ability of a single genotype to exploit the same range of niches, then migration potential of a species would be a deciding factor in determining whether a variety of host-specific or a single general purpose genotype is selected. Then it would seem that species with strong migration potential like Drosophila, would not show an association between genetic variation and niche-width. It is only in species with plenty of genetic variation and limited migration potential that we should expect a positive association. Since even a small amount of migration is sufficient to dilute the effect of natural selection, it is not surprising that niche-variation hypothesis has not been generally substantiated in organisms with strong migration potential.

(ii) Seasonal Changes in Genetic Variation:

One of the most powerful methods of showing natural selection is to show that the genetic variation responds to the cyclic changes in the environment. A classic case of this kind is that of inversion polymorphism in D. pseudoobscura

(Dobzhansky, 1943). Even though it has not been possible to associate the changes in inversion frequencies with any single factor in the environment with absolute certainity (Anderson et al., 1975), there is no doubt that these changes are caused by natural selection. During the last decade hundreds of species of both animals and plants have been studied electrophoretically but in very few cases temporal changes in gene and genotypic frequencies have been studied. In Drosophila several allozyme loci have shown temporal changes. These are G-6 pd, Pgm, Pgi, Est-6 and Idh in D. pavani (Kojima et al., 1972), Acph in D. mimica (Rockwood, 1969), Adh, Aph-2 and Est-6 in D. melanogaster (Anxolabehere et al., 1976), Pgm and Me in sibling species of D. pseudoobscura and D. persimilis (Dobzhansky and Ayala, 1973), Idh-1, Pgm-1 and Est-2 in D. mimica (Steiner, 1979) and Pgm in D. engyochracea (Steiner, 1979). However only two of these studies, that by Dobzhansky and Ayala (1973) and by Steiner (1979) have been done over a long enough period of time (a year or more) that the changes can be taken to be due to some significant factor of the environment. Outside Drosophila significant temporal changes have been shown to occur in populations of Daphnia pulex (Berger and Sutherland, 1978) and Daphnia magna (Hebert, 1974; Hebert and Ward, 1976). However the allozyme changes in D. magna are not cyclical and it is not clear if the changes are brought about by permanent ecological change in the habitat or by the synthetic appearance of superior genotypes (Hebert, 1974).

Considering the paucity of such evidence, the seasonal changes in M. rosae are much more significant as they appear to be cyclic. To summarize: (1) Three polymorphic loci (Est-4, SDH and the morph colour locus) show significant change and the changes at Est-4 are cyclic. (2) the changes at Est-4 are quite significant, up to 60% over a period of six months, and finally, (3) changes at Est-4 are consistently in the same direction in all populations. The changes during asexual generations are of course due to reproductive differences between different clones. To maintain stable polymorphisms these changes must be balanced by other changes which must occur during the sexual generation. However, it is not clear which of the many factors, nonrandom mating, gametic selection, zygotic selection, if any, plays the significant role during the sexual generation to bring about this balance. However, in view of the large cyclic changes observed at Est-4 locus it would appear that the gene pool of M. rosae has evolved to make use of the cyclic parthenogenetic breeding systems for adapting to cyclic seasonal changes in the environment.

(iii) Clonal Selection and Balance Polymorphism:

Because of the problem of segregational load associated with maintaining a large number of individual loci polymorphic, it has been proposed that selection acts on gene complexes rather than on individual genes (Lewontin, 1974). An outcome of this mode of selection would be generation of linkage disequilibrium between loci. This proposal has generated

a lot of interest in looking for non-random association of alleles at allozyme loci in a variety of organisms (Lewontin, 1974, Hedrick et al., 1978). The general finding seems to be that in sexually random breeding population like Drosophila, linkage disequilibrium between allozyme loci are relatively rare (Lewontin, 1974; Hedrick et al., 1978). We don't know if this is because most loci studied so far are loosely linked (Lewontin, 1974; Langley, 1977) or because the genes studied have been functionally unrelated (Zouros and Johnson, 1976), or simply because linkage disequilibriums in natural populations are indeed rare. So far the strong evidence of linkage disequilibrium has come from organisms which for one or another reason are more likely to show it. Thus Avena barbata, a predominantly selfing annual , has been shown to maintain strong linkage disequilibrium between five allozyme loci (Allard et al., 1972). The various gametic types are said to be strongly associated with the mesic or xeric micro habitat types (Allard and Kahler, 1974; Allard et al., 1972; Hamrick and Holden, 1978). The main problem with invoking selection for the observed nonrandom association of alleles in this species is that selfing, like linkage, has a retarding effect on the breakdown of linkage disequilibrium once it is generated. Also since the two Avena barbata ecotypes having different allelic combinations probably differ at many more loci, we do not know if the allozyme loci are themselves involved

in selection (Hedrick and Holden, 1978). Exactly the same arguments apply to the evidence in barley, Hardeum vulgare (Weir et al., 1972, 1974; Clegg et al., 1972; Allard and Kahler, 1972; Singh, 1972). Another interesting case of linkage disequilibrium in a nonrandom mating organism has been shown in Daphnia magna by Hebert and his associates (Hebert, 1974a,b; Hebert and Ward, 1976). They found non-random association between alleles at an Esterase and a Malate dehydrogenase locus. At one site these associations were relatively constant during the study period (Hebert, 1974a), while at another site there were large temporal changes in the association during the year the population was monitored (Hebert and Ward, 1976). It is not known if these two loci are linked.

With respect to breeding system, M. rosae and Daphnia magna are alike - both use cyclic parthenogenesis. Since during the asexual generation selection must act on the whole genome and not on single genes, large scale changes in the frequency of clonal types are not surprising. However, the effect of clonal selection would be to reduce the genetic variation in the population unless overdominance, cyclic selection or multiple niche polymorphisms are involved. Because M. rosae has only one host and because migration is presumably quite common, multiple niche polymorphism is out of the question. Similarly there is no evidence for overdominance at allozyme loci studied here. Thus by the process of elimination the

maintenance of polymorphism in this species appear to be mainly due to two factors: (1) cyclic temporal changes in genotypes in response to changes in the environment and (2) differing fitness values for the same gene or genotype during the sexual and asexual generations. In organisms like Daphnia and Aphids where sexual and asexual generations alternate, such adaptive adjustment of genotypic selective values between sexual, asexual generation is necessary. This is because during asexual generation the selection acts on clones and all genes of a clone, good or bad, get selected. This can only be counter-balanced during sexual generation through recombination and selection. This kind of selection is likely to generate linkage disequilibrium but the number of loci examined in this study are too few to provide any evidence. In this study, while at Est-4 locus the selection may be acting at the locus itself, but the morph colour locus itself may not be the site of selection as the same phenotype shows different pattern in different population. At a third locus Est-2 no temporal changes were observed. This would indicate that the gene pool of M. rosae consists of a limited number of clonal types made up of segregating gene blocks. The strong cyclic temporal changes within the year but overall stable frequency between years suggests that the fitness of various genotypes indeed vary during the sexual and asexual generations. From this it would seem that in M. rosae the breeding

system plays an active role in determining the mode of selection involved in the maintenace of balance polymorphism. Study of allozyme loci in other aphid species over space and time would provide evidence to untangle the role of breeding system, mode of selection and genetic background in the maintenance of genetic polymorphism.

REFERENCES

Allard, R.W., G.R. Babbel, M.T. Clegg and A.L. Kahler, 1972. Evidence for coadaptation in Avena barbata. Proc. Nat. Acad. Sci. USA 69:3043-48.

Allard, R.W. and A.L. Kahler, 1974. Multilocus genetic organization and morphogenesis. Brookhaven Symp. Biol. 225:239-243.

Anderson, W., T. Dobzhansky, O. Parlovsky, J. Powell and D. Yardley, 1975. Genetics of Natural Populations XLII. Three decades of Genetic Change in Drosophila pseudoobscura. Evolution 29:24-36.

Angus, R.A. 1980., Geographic dispersal and clonal diversity in unisexual fish populations. Amer. Nat. 115(4):531-550.

Anxolabehere, D., P. Girard, L. Palabost and G. Periquet, 1976. Stabilité des polymorphismes morphologique et enzymatique d'une population naturelle de Drosophila melanogaster. Arch. Zool. Exp. Gen. 117:169-179.

Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Mourao and S. Perez-Salas, 1972. Enzyme variability in the D. willistoni group. IV. Genic variation in natural populations of D. willistoni. Genetics 71:113-39.

Ayala, F.J. and J.F. McDonald, 1974. Genetic response to environmental heterogeneity. Nature 250:572-574.

Baker, J.P. 1978. Electrophoretic Studies on populations of Myzus persicae in Scotland from March to July 1976. Ann. appl. Biol. 88:1-11.

Baker, J.P. 1979. Electrophoretic Studies on populations of Myzus persicae in Scotland from October to December, 1976. Ann. appl. Biol. 91:159-164.

Beranek, A.P. 1974. Esterase Variation and Organophosphate resistance in populations of Aphis fabae and Myzue persicae. Ent. exp. and appl. 17:129-142.

Beranek, A.P. and R.J. Berry, 1974. Inherited changes in enzyme patterns within parthenogenetic clones of Aphis fabae. J. Entomol. 48:141-147.

Blackman, R.L., 1975. Photoperiodic determination of the male and female sexual morphs of Myzus persicae. J. Insect physiol. 21:435-53.

Charlesworth, B. and Charlesworth, D. 1979. A study of linkage disequilibrium in British populations of Drosophila subobsura. Genetics 92:983-994.

Clegg, M.T., Allard, R.W. and A.L. Kahler, 1972. Is the gene the unit of selection? Evidence from two experimental plant populations. Proc. Nat. Acad. Sci. U.S.A. 69:2474-2478.

Clegg, M.T. and R.W. Allard, 1972. Patterns of Genetic differentiation in the Slender Wild Oat Species Avena barbata. Proc. Nat. Acad. Sci. 69(7):1820-1824.

Dobzhansky, T. 1943. Genetics of natural populations. IX. Temporal changes in the composition of populations of Drosophila pseudoobscura. Genetics 28:162-186.

Dobzhansky, T. 1955. A review of some fundamental concepts and problems of population genetics. Cold Spring Harbour Symp. quant. Biol. 20:1-15.

Dobzhansky, T. and F.J. Ayala, 1973. Temporal frequency changes of enzyme and chromosomal polymorphisms in natural populations of Drosophila. Proc. Nat. Acad. Sci. U.S.A. 70:680-683.

Fuerst, P.A., R. Chakraborty and M. Nei, 1977. Statistical Studies on protein polymorphism in natural populations. I. Distribution of single locus heterozygosity. Genetics 86:455-483.

Furk, C. 1979. Field Collections of Aphis fabae scopoli S. Lat. (Homoptera:Aphididae) studied by starch gel electrophoresis and isoelectric focusing. Comp. Biochem. Physiol. 62B:225-230.

Grossman, A.I., L.G. Korevena and L.E. Ulitskaya, 1969. Variation of the alcohol dehydrogenase locus in natural populations of Drosophila melanogaster. Genetika 6:91-96.

Hamrick, J.L. and R.W. Allard, 1972. Microgenographical Variation in allozyme frequency in Avena barbata. Proc. Nat. Acad. Sci. 69(8):2100-2104.

Hamrick, J.L. and L.R. Holden, 1978. The influence of microhabitat heterogeneity on gene frequency distribution and gametic phase disequilibrium in Avena barbata. Evolution 33:521-533.

Harris, H. and D.A. Hopkinson, 1975. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland publishing Company, Amsterdam. Oxford American Elsevier Publishing Company Inc., N.Y.

Hebert, P.D.N. 1974. Ecological differences between genotypes in a natural population of Daphnia magna. Heredity 33:327-337.

Hebert, P.D.N. 1974b. Enzyme variability in natural populations of Daphnia magna III. Genotypic frequencies in intermittent population. Genetics 77:335-341.

Hebert, P.D.N. 1974a. Enzyme variability in natural populations of Daphnia magna II. Genotypic frequencies in permanent populations. Genetics 77:323-334.

Hebert, P.D.N. and R.D. Ward, 1976. Enzyme variability in natural populations of Daphnia magna IV. Ecological differentiation and frequency changes in genotypes at Audley End. Heredity 36:331-341.

Hedrick, P.S., S. Jain and L. Holden, 1978. Multilocus systems in evolution. Evol. Biol. 11:101-184.

Hedrick, P.W., M.E. Ginevan and E.P. Ewing, 1976. Genetic polymorphism in heterogeneous environments. Ann. Rev. Ecol. Syst. 7:1-32.

Jaenike, J. and R.K. Selander, 1979. Ecological generalism in Drosophila falleni: Genetic evidence. Evolution 33:741-748.

Jaenike, J., E. Vavis Parker, Jr. and R.K. Selander, 1980. Clonal Niche Structure in the parthenognetic earthworm Octolasion tyrtaeum. Amer. Nat. 116: 196-205.

Kenten, J. 1956. The effect of photoperiod and temperature on reproduction in Acyrthosiphon pisum (Harris) and on the forms produced. Bull. ent. Res. 46:599-624.

Kimura, M. 1968. Evolutionary rate at the molecular level. Nature 217:624-26.

Kimura, M. and T. Ohta, 1971. Theoretical Aspects of Population Genetics. Princeton University Press.

Kimura, M. and T. Maruyama, 1971. Pattern of neutral polymorphism in a geographically structured population. Genet. Res. 18:125-131.

Koehn, R.K. and D.J. Rasmussen, 1967. Polymorphic and monomorphic serum esterase heterogeneity in catastomid fish population. Biochem. Genet. 1:131-144..

Langley, C.H., Y.N. Tabori and K. Kojima, 1979. Linkage disequilibrium in natural populations of Drosophila melanogaster. Genetics 78:921-936.

Langley, C.H., R. Ito and R.A. Voelker, 1977. Linkage disequilibrium in natural populations of Drosophila melanogaster. Seasonal variation. Genetics 86:447-454.

Langley, C.H. 1977. Nonrandom associations between allozymes in natural populations of Drosophila melanogaster. In: F.B. Christiansen and T.M. Fenchel (eds.). Measuring Sélection in Natural populations. Springer Verlag, N.Y.

Lees, A.D. 1959. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid Megoura viciae Buckton. I. The infleunce of these factors on apterous virgino -parae and their progeny. J. insec. Physiol. 3: 92-117.

Lewontin, R.C. 1974. The Genetic Basis of Ecolutionary Change. Columbia Univ. press, N.Y.

Lokki, J., A. Saura, P. Lankinen and E. Suomalainen, 1976. Genetic polymorphism and evolution in parthenogenetic animals.V. Triploid Adoxus obscurus (Coleoptera: Chrysomelidae). Genet. Res. Camb. 28:27-36.

Maelzer, D.A. 1977. The biology and main causes of Change in number of the rose aphid Macrosiphum rosae (L) in Adelaide, South Australia. Aust. J. Zool. 25: 269-284.

May, B. and F.R. Holbrook, 1978. Absence of genetic variability in the Green Peach Aphid, Myzus persicae (Hemiptera:Aphididae). Ann. entomol. Soc. Am. 71:

809-812.

Mitter, C. J. Douglas, J.C. Schneider and J. Daneil Hare, 1979. Genetic variation and host plant relations in a parthenogenetic moth. Evolution 33:777-789.

Morton, R.A. and R.S. Singh, 1980. Variation in Drosophila Acetylcholine Esterase. Biochem. Genet. 18:439-454.

Mukai, T., T.K. Watanabe and O. Yamaguchi, 1974. The genetic structure of natural populations of Drosophila melanogaster. XII Linkage disequilibrium in a large local population. Genetics 77:771-793.

Mukai, T. and R.A. Veolker, 1977. The genetic structure of natural populations of Drosophila melanogaster. XIII. Further Studies on Linkage Disequilibrium. Genetics 86:175-185.

Nei, M. 1975. Molecular Population Genetics and Evolution. American Elsevier Inc., New York.

Nevo, E., 1978. Genetic variation in natural populations: patterns and theory. Theor. pop. Biol. 13:121-177.

Nur, U. 1977. Electrophoretic comparison of enzymes of sexual and parthenogenetic mealybugs (Homoptera: Coccoidea:Pseudococcidae). Va. Polytech. Inst. State Univ. Res. Div. Bull. IV:127:69-84.

O'Gower, A.K. and P.I. Nicol, 1968. A latitudinal cline of hemoglobins in a bivalve mollusc. Heredity 23: 485-92.

Ohta, T. 1974. Mutational pressure as the main cause of molecular evolution and polymorphism. Nature 252: 351-354.

Powell, R.J. 1971. Genetic polymorphisms in varied environments. Science 174:1035-1036.

Powell, J.R., 1975. Protein variations in natural populations of aniamls. in "Evolutionary Biology". (Th. Dobzhansky, M.K., Hecht and W.C. Steera, eds.) 8:79-119.

Prakash, S. and R.C. Lewontin, 1968. A molecular approach to the study of genic heterozygosity. III. Direct evidence of co-adaptation in gene arrangements of Drosophila. Proc. Nat. Acad. Sci. U.S.A. 59:398-405.

Prakash, S. and R.C. Lewontin, 1971. A molecular approach to the study of genic heterozygosity. V. Further direct evidence of co-adaptation in inversion of Drosophila. Genetics 69:405-408.

Rockwood, E.S. 1969. Enzyme variation in natural populations of Drosophila mimica. Studies in Genetic V. Tex. Univ. Publ. 6918:111-25.

Sabath, D.M. 1974. Niche breadth and genetic variability in sympatric natural populations of Drosophilia flies. Amer. Nat. 108(962):533-540.

Schneider, J.C. 1978. Phenological and kentromorphic aspects of the population ecology of the fall cankerworm Alsophila pometaria Harris (Lepidoptera: Geometridae) Ph.D. Dissertation, Princeton Univ., Princeton.

Scudday, J.F. 1973. A new species of lizard of the Cremidophorus stesselatus group from Texas. J. Herpetol. 7:363-371.

Selander, R.K. 1976. Genic Variation in Natural Populations. Molecular Evolution (F. Ayala, Ed.) 21-45. Sinauer Assoc. Inc., Suderland, Mass.

Shaw, C.R. and R. Prasad, 1970. Starch gel electrophoresis of enzymes - A compilation of recipes. Biochem. Genetics 4:297-320.

Shugart, Jr. and B.G. Blaylock, 1973. The niche-variation hypothesis: An experimental study with Drosophila populations. Amer. Natur. 107(956):575-579.

Singh, R.S. 1972. Genetic variability and selective forces in two bulk-hybrid populations of barley. Ph.D. Thesis, Univ. California, Davis.

Smith, M.Y. and A. Fraser, 1976. Polymorphism in a cyclic parthenogenetic species Simocephalus serrulatus. Genetics 84:634-637.

Soule, M. and B. Stewart, 1970. The "niche-variation" hypothesis: A test and alteratives. Amer. Natur. 104(935):85-97.

Steiner, W.W.M. 1977. Niche width and genetic variation in Hawaiian Drosophila. Amer. Nat. 111(982):1037-1045.

Steiner, W.W.M. 1979. Genetic variation in Hawaiian Drosophila VI. Seasonally-dependent gene changes in Drosophila mimita. Evolution 33:543-562.

Steiner, W.W.M. 1980. On niche width and genetic variation, parametrical problems, and gene pool differentiation in Drosophila. Am. Nat. 115: 596-599.

Stille, B., H. Ochman and R.K. Selander, 1980. Genetic Structure of populations of the earthworm Aporrectodea tuberculata. Oikos 34:195-201.

Sudderuddin, K.I. 1973. An electrophoretic study of some hydrolases from an Op-susceptible and an Op-resistant strain of the green peach aphid Myzus persicae (Sulz.). Comp. Biochem. physiol. 44B:923-929.

Suomalainen, E., A. Saura, J. Lokki and T. Terri, 1980. Genetic polymorphism and Evolution in parthenogenetic animals. Theor. Appl. Genet. 57:129-132.

Takada, H. 1979. Characteristics of forms of Myzus persicae (Sulzer) (Homoptera:Aphididae) distinguished by colour and esterase differences and their occurrence in populations on different host plants in Japan. App. Ent. Zool. 14(4):370-375.

Tomiuk, J. and K. Wohrmann, 1980. Enzyme variability in populations of aphids. Theor. appl. Genet. 57: 125-127.

Van Valen, L. 1965. Morphological variation and the width of the ecological niche. Amer. Natur. 99:377-389.

Vrijenhoek, R.C. 1978. Coexistence of clones in a Heterogenous environment. Science 199:549-552.

Weir, B.S., R.W. Allard and A.L. Kahler, 1972. Analysis of complex allozyme polymorphisms in a barley population. Genetics 72:502-523.

Weir, B.S., R.W. Allard and A.L. Kahler, 1974. Further analysis of complex allozyme polymorphisms in a barley population. Genetics 78:911-919.

Wool, D., S. Bunting and H.F. Van Emden, 1978a. Electrophoretic study of genetic variation in British Myzus persicae (Sulz.) (Hemiptera, Aphididae). Biochem. Genetics 16:987-1006.

Wool, D., H.F. Van Emden and S.W. Bunting, 1978b. Electrophoretic detection of the internal parasite, Aphidus matricariae in Myzus persicae. Ann. appl. Biol. 90:21-26.

Young, J.P.W., 1979. Enzyme polymorphism and cyclic parthenogenesis in Daphnia magna I: Selection and clonal diversity. II. Heterosis following sexual reproduction. Genetics 92:953-982.

Zouros, E. and W. Johnson, 1976. Linkage disequilibrium between functionally related enzyme loci of Drosophila mojavensis. Can. J. Genet. Cytol. 18:245-254.

Berger, E. and J. Sutherland, 1978. Allozyme Variation in two Natural Populations of Daphnia Pulex. Heredity 41:13-23.

Kojima, K., P. Smouse, S.Y. Yang, P.S. Nair and D. Baneic 1972. Isozyme frequency patterns in Drosophila pavani associated with geographical and seasonal variables. Genetics 72:721-731.

Prakash, S., R.C. Lewontin and J.L. Hubby, 1969. A molecular approach to the Study of genic heterozygosity in natural populations. IV. Patterns of genic variation in Central, marginal and isolated populations of Drosophila pseudoobscura. Genetics 61:841-58.