Variability in Migratory Tendency Within and Among Natural Populations of the Pea Aphid, *Acyrthosiphon pisum*

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Summary. The migratory tendencies of pea aphids were measured by determining the numbers of winged and non-winged offspring produced by parthenogenetic wingless females after a crowding test. Sources of variability in this measure were investigated. The migratory tendency of an individual clone was found to be stable. Spatial and temporal patterns in migratory tendency were found among nine natural populations. These patterns probably reflect differences in the frequencies of a large number of genetically distinct clones. Hypotheses based on the relative fitness of immigrant and resident clones and the heritability of migratory tendency are offered to account for these results. High migration rates may be required to account for genetic differentiation within and among some parthenogenetic populations of the pea aphid.

Introduction

Many species of aphids exhibit a seasonal pattern of alternating sexual and asexual generations. Typically, the single annual sexual generation produces overwintering eggs and has a rather low reproductive output. The summer viviparous, parthenogenetic generations exhibit very high reproductive rates (Kennedy and Stroyan, 1959). This life history pattern has been viewed as exploiting the advantages of both sexuality and parthenogenesis (Dobzansky et al., 1977, pg. 132). Parthenogenesis in aphids appears to allow for no recombination (Blackman, 1978), but permits the rapid reproduction of well-adapted genotypes. The sexual generation provides the opportunity for recombination and the synthesis of new genotypes.

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While the consequences of this reproductive strategy to the ecology and microevolution of aphids has rarely been studied, Hebert (1974) has investigated the process in the freshwater crustacean, *Daphnia magna,* which has a similar breeding system. Certain populations of *Daphnia* and aphids inhabit environments with analogous spatial characteristics. The pond habitats of *Daphnia* and the agricultural fields inhabited by some aphids are both patchily distributed with large distances between suitable habitats. Such distances can only be traversed by a specially adapted dispersing stage. Hebert (1974) has concluded that the local genetic differentiation he observed among *Daphnia* populations results from their low vagility and the resulting low gene flow among the populations. Here the similarity betwen *Daphnia* and aphids breaks down since aphids produce winged forms which readily travel long distances and are able to rapidly colonize habitats. Therefore, local populations of aphids might not be expected to show genetic differentiation.

The pea aphid, *Acyrthosiphon pisum* (Harris), appears to have a high migratory rate. It is a common species in southern Ontario living on a number of wild and cultivated legumes, but it is particularly abundant on alfalfa, *Medicago sativa* L. It overwinters in this crop as a sexually-produced egg. The females which hatch from the eggs are the first of a series of summer parthenogenetic generations. In the fall, photoperiod and temperature stimulate the parthenogenetic females to produce a single sexual generation (Lamb and Pointing, 1972). Sexual females are never winged and males are rarely winged so that any migration or mixing of local populations occurs during the parthenogenetic part of the life history. Winged forms or alatae are abundant through the late spring and summer. On alfalfa we have found that more than 40% ($N=1251$) of the larvae have wing buds. Similar levels of winged form production have been observed by Dunn and Wright (1955) in England. Newly planted alfalfa fields are colonized soon after germination providing a suitable habitat for up to five years. Pea fields are planted in rotation with non-host crops. They are rapidly and repeatedly invaded to an extent that a number of applications of insecticide may be required to control aphid damage. The persistence of wild hosts is unknown, but they are perennial.

The physiological mechanisms controlling the production of alatae have been well studied. Sutherland (1969a) has shown that physical contact among adult pea aphids or a simulated crowding experience initiates alata production. Deterioration of the host plant has a similar effect (Sutherland, 1969b). The intensity of the response is modified by a maternal age effect (MacKay and Wellington, 1977; MacKay, 1977), such that age structure phenomena may alter the response of natural populations.

We expected that high migration rates during the parthenogenetic phase would result in constant mixing of local populations and limit genetic differentiation among them. However, in conjunction with research on the ecological significance of variability in migratory tendency in populations of differing ages (MacKay and Lamb, 1979), we found what appeared to be genetic differences among nearby populations. The present study was designed to assess whether the differences were indeed genetic and to characterize the variability in migratory tendency within and among natural populations of the pea aphid.

The term "migratory tendency" is a convenient one. It stresses the fact that the number of alatae produced by a female or by a population is affected not only by the immediate environmental conditions (e.g. population density or host plant condition), but also by inherent qualities of the individuals in a population. The inherent quality of individuals may result from a physiological mechanism such as a maternal age effect or from their genotype. For the pea aphid, a migratory tendency can be defined for an individual female, a clone, or a population. In each case, it is the intensity of response to a standardized stimulus measured as the percentage of alatae the individual, clone, or population produces among its offspring.

Materials and Methods

Variation was assessed by measuring the migratory tendency of parthenogenetic individuals selected from a series of laboratory clones. The clones were obtained from sweep samples of pea aphids taken from alfalfa (*Medicago sativa* L.) fields in Essex County, Ontario, Canada, Nine fields (A, B, C, ..., I) were sampled, most only once, some twice, and one 4 times. The sampling occurred in four intervals during the summer of 1977. The timing of the sampling intervals and the number of clones collected per field were determined by our capacity to process families of aphids in the laboratory. In the early June and late June samples, 40 clones were collected per field and no replicate tests were made on individual clones. Only 10 clones were collected from each field in the mid-July and mid-August samples, but 10 tests were made on each of the clones. To assure that the clones were representative of the population in each field, each sweep sample was taken from a different part of the field, excluding a 2 m band around the edge where recent immigrants were likely to be most common (Lewis, 1969). From each sample one uninjured apterous adult aphid was retained.

The offspring produced by each field-collected adult female during its first 1–2 days in the laboratory were designated the first laboratory generation for each clone. Their offspring were designated the second laboratory generation. All clones were reared on individual excised broad bean (*Vicia faba* L.) leaves on moistened paper towel in 60-mm diam. petri dishes. Undamaged leaves were selected from plants in the eight-leaf stage. Neither the top nor bottom pair of leaves were used. Plants were grown in a perlite-vermiculite mixture and watered with a nutrient solution. Plants and aphids were reared at room temperature which varied between 20 and 25° C with a 16 h light cycle (lights on at 07:00).

Migratory tendency was defined as the percentage of alatae among the offspring of a laboratory reared female after she had been subjected to a standard stimulus. This stimulus was a modified version of that used by Sutherland (1969a) and consisted of a simulated crowding test where 10 apterous adult aphids wandered freely on moistened paper towel in a 60-mm diam. petri dish for 20 h starting at 15:00 h. The 10 aphids were made up of either females from different clones or 10 sisters. The crowding dishes were held in an environmental chamber at $20 \pm 1°$ C and 16 h light. Each female was reared individually from late in its fourth instar and allowed to produce offspring for about 24 h before being tested. The reproductive age of each female at the beginning of the test was measured by the number of pre-crowding offspring. These larvae were reared as a group to the fourth instar when the percentage of alatae in each batch could be determined. After being crowded, each aphid was placed individually on a fresh excised leaf and allowed to reproduce for 30 h. The effect of the crowding stimulus wanes after this period. After 30 h of reproduction, the adult was discarded and the post-crowding batch of larvae was reared as a group to the fourth instar when the percentage of alatae could be determined.

To minimize or control the variability due to known characteristics of the physiological mechanism controlling wing determination, test aphids were reared in isolation from the fourth instar. Only adults of *A. pisum* are sensitive to crowding (Sutherland, 1969a). Also, the quality of the excised leaves was standardized and then leaves were assigned at random to the aphids. To control for maternal age effects, only aphids of the second laboratory generation were tested and these

were selected from larvae born in the first 1–2 days of the reproductive period. Since field collected aphids were of variable and unknown ages, the aphids of the first laboratory generation consisted of an unknown mixture of early- and late-born individuals and were therefore unsuitable for testing (MacKay and Wellington, 1977).

Because the crowding stimulus for each aphid is the result of a stochastic process dependent on the number of interactions between aphids and because the % of alatae must be determined from a small sample, the index of migratory tendency is inherently variable apart from any underlying genetic variation. To reduce the effect of this variability and assure that the data met the assumptions of standard statistical procedures, the results for several females were often pooled. Pooling of results was done in two ways. When replicates for one clone were available, all such replicates (usually 10) were pooled. If there were no replicates for a clone, then the results from one crowding group (10 aphids from an unknown number of different clones) were pooled. The pooling procedure guaranteed that percentages used in statistical analyses were based on a sample of about 150 aphids, except in one experiment where groups of sisters were marked and mixed. In this case, each percentage was based on a sample of 75 aphids. All percentages were subjected to an arcsin transformation before being used in analyses of correlation (Sokal and Rohlf, 1969).

Results

The pre-crowding batch of offspring averaged 12.3 ± 4.3 (S.D., $N = 198$) larvae. As well as indicating the reproductive age of each test female, the pre-crowding batch provided a second measure of migratory tendency: the response of the parent to the condition of a standardized and randomly assigned leaf. However, since the pre-crowding batch size was smaller and relatively few alatae were produced (20.6%, $N = 4,701$), the measure was more variable than the measure based on the crowding test and will not be discussed in detail. The post-crowding batch averaged 14.2 ± 3.0 (S.D., $N = 198$) larvae of which 66.2% ($N = 11,620$; 815 batches) were alatae.

Within- and Among-clone Variation

The percentage of alatae in the individual post-crowding batches of offspring varied from 0 to 100%. The distribution of these percentages for 473 clones tested is illustrated in Fig. 1 A. It resembles a truncated normal distribution except for the excess of very low percentages. The modal class is 90–100%. The distribution is similar for the 38 clones for which the estimates of migratory tendency are based on 10 pooled replicates per clone (Fig. 1 B). All these clones produced both alatae and apterae so that no 0 or 100% values occur after pooling and the distribution more closely approximates a truncated normal. The figures show that the variability in migratory tendencies among clones was very great even when environmental conditions were carefully controlled and each estimate was based on a sample of about 150 offspring.

While many sources of environmental variation were controlled, there are still some possible environmental explanations for the observed variability. For example, only young adult apterous females were tested, but the reproductive age of females (as reflected by the size of their pre-crowding batch) varied slightly at the time of the tests. However, no significant correlation could be found between pre-crowding batch size and migratory tendency ($r = 0.19$, $P =$
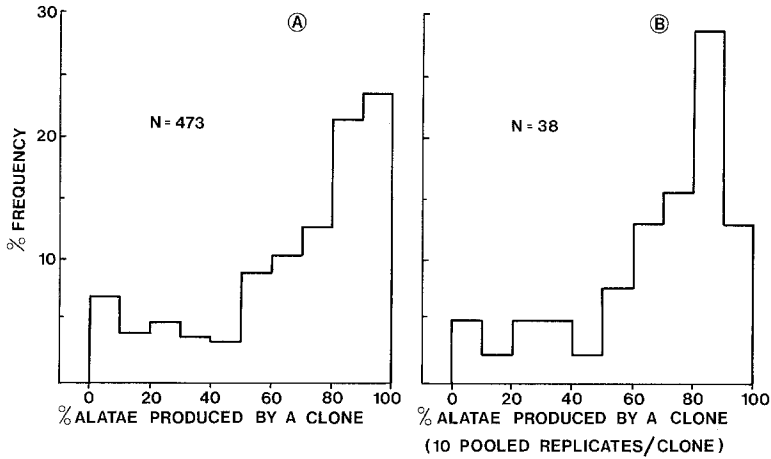
Fig. 1. The distribution of migratory tendencies measured as the % alatae produced by a female or clone after a crowding stimulus. Figure 1 A illustrates the distribution for most clones tested (*N*=473). Each % is based on approximately 15 offspring. Figure 1 B illustrates the distribution for all clones for which 10 replicate tests were conducted on each clone (*N*=38). Each % is based on approximately 150 offspring

0.26, *N*=38). Similarly, the size of the post-crowding batch varied somewhat. Since the response to the crowding stimulus is temporary, large batches might have contained more apterae than small batches. However, no significant correlation could be found between post-crowding batch size and migratory tendency (*r*= −0.18, *P*=0.29, *N*=38). Variation was also observed among replicates of the same clone (Table 1), but at a lower level than represented in Fig. 1 A. Since in many experiments, members of the same clone were crowded together, the similarity between replicates for each clone could have been due to a clone effect or a crowding-group effect. To distinguish these effects aphids from different clones were marked and mixed. Twenty such crowding tests were conducted, each with 5 sisters from one clone and 5 from another. Two tests were conducted per clone, but the same two clones were not paired in each test. We expected that if there was a crowding group effect, the migratory tendencies of the two clones of a group would be positively correlated. However, the correlation was negative and non-significant (*r*= −0.39, *P*=0.09, *N*=20).

Despite the variation in response among the members of a single clone, clones were consistent in their responses and showed characteristic response levels (Table 1). This observation is confirmed by the significant positive correlation (*r*=0.77, *P*=0.001, *N*=20) which was observed for the independent groups of replicates from the same clone (Fig. 2). Also, the response of clones to the crowding stimulus was found to be stable over time. Two clones, one exhibiting a low and the other a high migratory tendency, were maintained in the laboratory and tested three times over the period of the field sampling (Table 2). One clone showed no significant difference between tests and a range of 7.5% in migratory tendency. The clone with a low migratory tendency exhib-

Table 1. The migratory tendencies of 10 sisters (replicates) per clone from a sample of 4 of the 511 clones tested. These illustrate the within-clone variability for the characteristic responses to a crowding test. For each clone, two independent crowding tests (1 and 2) were performed. In each test, 5 sisters were crowded with 5 sisters from a different clone

Clone	Response	Response, % alatae per replicate										N, per replicate	
		Crowding test 1					Crowding test 2					mean	range
1	weak	0	0	0	0	0	0	0	0	0	7	12	7–14
2	medium	19	38	42	54	60	33	50	53	76	83	15	12–19
3	strong	81	85	93	100	100	58	71	73	100	100	14	10–17
4	most variable	0	0	21	33	45	47	53	67	86	89	19	15–22
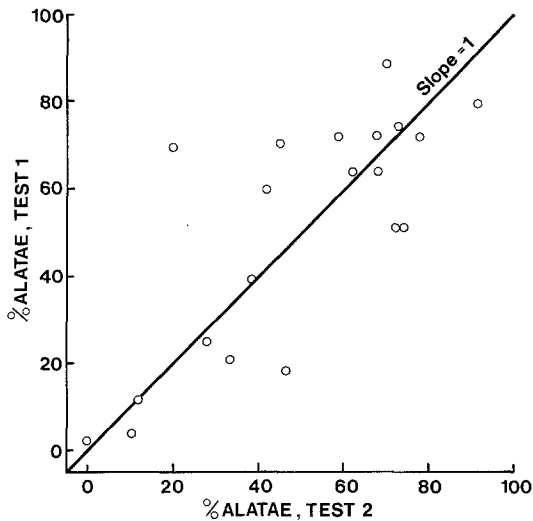
Fig. 2. The relation between two independent tests of migratory tendency for 20 clones. Each point is based on the results for two groups of 5 marked females (approximately 75 offspring) from one clone crowded with two groups of 5 marked females from different clones

Table 2. Variation in the % alatae produced by two clones during the period of the field sampling. Ten sisters were tested per clone on each day. A within-clone G-test for independence shows that only one result[a] differs at $P < 0.05$ from the other two

Test date	Clone 1		Clone 2	
	% alatae	N	% alatae	N
May 31	74.4	121	24.8	133
July 29	81.9	116	30.6	160
August 16	77.6	192	15.8[a]	183

ited a greater range (14.8%) but only one test gave results that differed significantly from the other two. This difference was due to a large number of families with no alatae in the one test. At low migratory tendencies, the number of families with no alatae can vary more widely resulting in larger apparent differences in pooled estimates. Further evidence that migratory tendency is characteristic of the clone is provided by a comparison of the response of clones to two different stimuli, leaf quality and crowding. The effects of leaf quality and crowding are reflected in the pre-crowding and post-crowding batches, respectively. The percentages of alatae produced in the two batches were positively correlated ($r=0.546$, $P=0.0004$, $N=38$).

Within- and Among-Population Variation

Table 3 illustrates the types of differences in migratory tendencies found within and among populations. Within each population, some members of the sample of 8–10 clones differed from the others. On both sampling dates, Field A yielded more clones with high migratory tendencies. A comparison of the pooled morph frequencies for all clones in a sample confirms that there were differences among the samples. Aphids from Field A produced more alatae than those from Fields H or F on the corresponding days (A-85.2% vs. H-81.0% and A-61.4% vs. F-40.6%). In addition, the clones collected in Field A on July 15 had a higher migratory tendency than clones collected in the same field on August 12. A G-test for independence (Sokal and Rohlf, 1969) shows that all the pooled frequencies differ significantly ($P<0.01$, $N=1009$ to 1581).

The spatial relationships of the fields and the average migratory tendency of each population sampled between June 3 and 7 is shown in Fig. 3. Populations A and B at the periphery of the agricultural area of the county have significantly lower migratory tendencies than the central populations (Table 4). Similar results were obtained for populations sampled between June 23–30 (Table 4). The

Table 3. The variation in migratory tendency among clones sampled from three fields on two dates. Each % is based on 10 tests per clone (approximately 150 offspring). Clones that do not differ based on a G-test for independence at $P<0.05$ are linked by a line

Field	Sample date	% Alatae produced by each clone									
		Clone number									
		1	2	3	4	5	6	7	8	9	10
A	July 15	95	95	88	85	84	81	81	80	79	79
H	July 15	94	92	90	89	84	80	72	53		
A	August 12	86	79	75	74	70	66	52	44	39	28
F	August 12	66	63	62	62	59	31	27	12	7	1
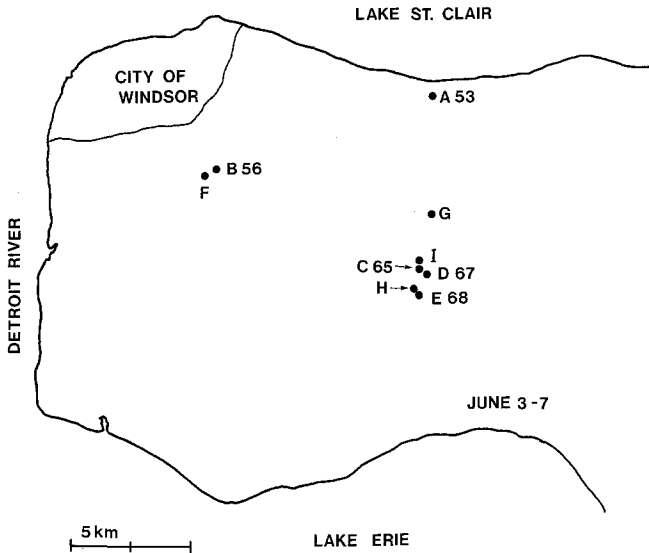
Fig. 3. Map of Essex County, Ontario, Canada showing the location of fields sampled. The % alatae produced after a crowding stimulus by 40 clones sampled from each population between June 3 and 7 is given beside the letter which designates each field

Table 4. A comparison of the % alatae produced by populations during two sampling periods in June. For each field, 37 to 40 clones were tested. Results that do not differ at $P < 0.05$ using a G-test for independence are linked by a line

	Field (date sampled)					
	A(3)	B(3)	C(7)	D(7)	E(3)	
% alatae	53.3	55.7	65.3	66.9	68.0	
N	646	662	542	577	556	
	Field (date sampled)					
	A(23)	F(23)	G(30)	H(27)	E(27)	I(30)
% alatae	68.5	69.1	69.8	74.0	75.8	77.4
N	527	517	517	603	504	540

peripheral populations A, B, and F are located near urban areas or water, and thus may receive migrants from only one direction. A strong seasonal pattern in the average migratory tendency of the populations was also observed (Fig. 4). The increase in migratory tendency from early June to a peak on July 15, followed by a lower value on August 12, was observed for Field A, which was sampled on four dates, as well as by the less frequently sampled fields.
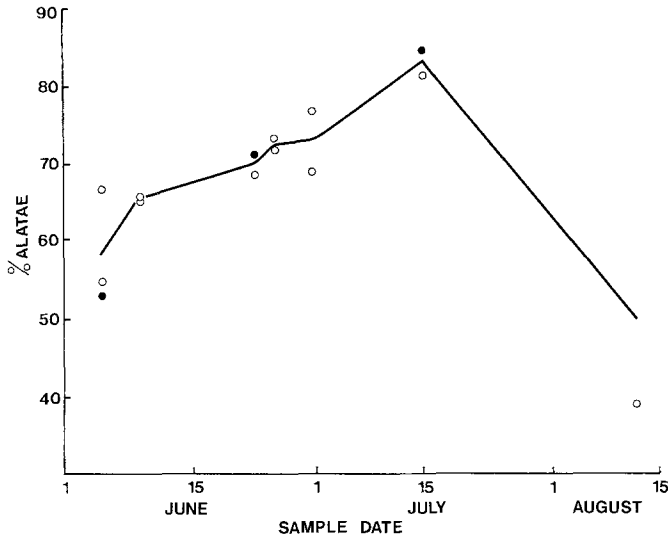
Fig. 4. The relation between migratory tendency of populations and sampling date. Repeated samples from Field A are represented by (●). Results for other fields for which only one or two samples are available are represented by (○)

Discussion

The migratory tendency of pea aphids, measured as the percentage of alatae in a post-crowding batch is highly variable. Part of this variability results from the stochastic nature of the stimulus, an indeterminate number of interactions between the test aphid and 9 others. Part also results from the fact that the % must be based on a sample of from 5 to 25 offspring. This latter source of variability can be reduced by pooling the results from families that were treated similarly and determining a migratory tendency for a clone or a migratory tendency for a sample from a population of clones. On pooling such data, it is clear that there is still much variability in migratory tendency that cannot be accounted for by these two sources which are inherent in the process and testing system.

This variability cannot be accounted for by any known physiological correlate since these are either controlled, excluded, or tested. For example, pre- and post-crowding batch size, and the composition of the crowding group do not account for a significant part of the variability. Leaf quality was standardized and maternal age was kept constant. These observations suggest that the remaining variation may be largely due to genetic differences among clones in their responsiveness to the environmental stimuli known to cause wing formation. This conclusion is supported by the positive significant correlation between migratory tendencies measured in response to a leaf stimulus (pre-crowding batch) and to a crowding stimulus (post-crowding batch). Further support for a large genetic component in this variation is provided by our observation of the temporal stability of the response in two clones. We also observed a highly significant positive correlation between independent determinations of

the migratory tendencies of groups of sisters. Therefore, the observed differences between samples from natural pea aphid populations probably reflect differences in the proportions of many genetically distinct clones within the populations.

Our samples from different fields indicate that there were significant differences among populations less than 20 km apart. However, fields that were very close had populations with similar migratory tendencies. The data are too limited to permit any firm conclusion on geographical patterns in migratory tendency. However, our results are consistent with the hypothesis that populations at the edge of this partially isolated area of alfalfa fields have lower tendencies to migrate. Essex County has large bodies of water on the north and south and on the west is a large river and even wider barrier of urban and industrial land. It seems likely that these areas absorb emigrants but provide no sources of immigrants. The result may be a lower ratio of immigrants to emigrants and a greater loss of the genes for migratory tendency in populations at the edges of the county than in populations which have sources of immigrants at all points of the compass.

Migratory tendency peaked in mid-July at a level about 40% higher than migratory tendencies measured a month earlier or later. This pattern is correlated with at least two other events in the life history of the species. Pea aphid populations often decline dramatically in July. We had much more difficulty collecting 40 healthy adult females per field at this time than in June (see also Dunn and Wright, 1955; and Campbell and Mackauer, 1977). The largest flight of alate pea aphids occurs in mid-June about a month before the peak in migratory tendency of the population. W.M. Elliott (unpublished data collected at the Agriculture Canada Research Station), using a suction trap at Harrow in Essex County in the years 1967–1976 found that on average the largest catch of alatae occurred in the week ending June 18.

The rise in migratory tendency in early summer may be due to an influx of immigrant clones which have a higher migratory tendency than the resident population because they are the offspring of females that responded to the stimuli which cause wing production. The immigrants also seem to have a higher fitness than the residents which leads to an increase in their frequency in the population and a dramatic increase in migratory tendency. Aphid populations are declining rapidly at this time probably because mortality and emigration rates are both high. One temporary advantage immigrants have over resident clones is that immigrants are prevented from producing alatae that may be lost to the population. A maternal age effect restricts the capacity of alatae and their first-born progeny for producing alatae (MacKay, 1977). This could lead to a higher fitness among immigrants at a time of high levels of crowding and subsequent dispersal. The drop in migratory tendency later in summer may also be accounted for by differential migration rates between immigrants and residents. Since migratory tendency is inherited a larger proportion of the immigrant clones would be lost as emigrants once the lag period caused by maternal age effects had elapsed. If this explanation for the decline in migratory tendency is true, there may be little effective immigration into established populations. The immigrant clones which appear to dominate the populations in summer may have disappeared from the alfalfa fields by fall.

These explanations for the spatial and temporal variation in migratory tendency are offered as hypotheses that are consistent with the data currently available. They rely primarily on three assumptions. Migratory tendency is assumed to be higher among the progeny of emigrants or immigrants than longer-term residents, i.e. the heritability of migratory tendency is high. Secondly, the fitness of clones with high migratory tendency is lower in isolated populations. Finally, the clones with a high migratory tendency are assumed to exhibit a higher fitness during the early summer when populations of the pea aphid are declining, possibly because they produce fewer emigrants due to temporary physiological restrictions. Perhaps of more interest than the explanations themselves is the fact that an explanation is required. In the face of apparently high migration rates, spatial and temporal differences in migratory tendency occur over short distances and within a single season. For the pea aphid, high migration rates coupled with differences in fitness between resident and immigrant clones may account for the observed patterns of migratory tendency.

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