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Clonal diversity, population structure, and dispersal in the parthenogenetic moth *Ectoedemia argyropeza*

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Abstract

Populations of the parthenogenetic moth *Ectoedemia argyropeza* (Lepidoptera, Nepticulidae) were studied for their clonal composition. Clones were characterized by 6 polymorphic enzyme loci. In a geographic survey 32 clones were observed among 812 individuals. Two clones were predominant, together they explained over 75% of the total variation. Relationships among clones hinted at a monophyletic origin of the species. Analysis of the life cycle and population structure indicated that *E. argyropeza* is a very sedentary species, with bottlenecks, drift, and passive migration as important population genetical factors moulding the variation.

Introduction

Until recently asexual species were assumed to possess little genetic variation (Williams, 1975; Case & Taper, 1986). Following from Fisher's (1930) fundamental theorem of natural selection the evolutionary potential of a species is a direct function of the amount of its genetic variation. Genetically diverse offspring provide the raw material for selection of better adapted genotypes. Thus it has been claimed that parthenogenesis constitutes a dead end of evolution because parthenogenetic organisms cannot adapt to changing environments (Darlington, 1932; Fisher, 1958; Went, 1984). Suomalainen (1961) was the first who challenged the validity of this claim by revealing large amounts of morphological variation among populations of polyploid apomictic weevils; he assumed a genetic basis for the morphological variation, implying considerable differences in their gene pools, and indicating they were still capable of evolution.

Up to the mid-sixties suitable methods to study

the genetics of parthenogenetic species were largely lacking and consequently most studies were theoretical by nature. Enzyme electrophoresis provided the tools to elucidate single-gene polymorphisms, to ascertain the genetic composition of populations, and to determine the relationships among bisexuals and their derivative diploid and polyploid parthenogenetic forms. Electrophoresis has revealed much more genetic diversity than was formerly supposed to be present in populations of parthenogenetic species (for insects see Suomalainen *et al.*, 1976).

Parthenogenesis can be divided into 3 major types: apomictic (or ameiotic), automictic (or meiotic), and generative or haploid parthenogenesis (the common mode of reproduction in Hymenoptera). Apomixis, the most common mode in insects, leads to offspring that retain the genetic constitution of the mother, barring mutation. In theory mutation pressure steadily increases genetic diversity such that beneficial mutations accumulate at the lowest and harmful mutations at the highest rate as compared

to biparental, selfing, and automictic organisms (Pamilo *et al.*, 1987). In automictic parthenogenesis, homozygosity usually replaces heterozygosity. This especially pertains to gamete duplication (*e.g.*, Stille, 1985). Although sexual reproduction is the primary mode, a good many species of insects reproduce by parthenogenesis, which always represents the derived condition (Suomalainen *et al.*, 1976).

Ectoedemia argyropeza (Zeller) is a univoltine moth belonging to the Nepticulidae and is widely distributed in Europe. It is strictly monophagous on *Populus tremula* and the only *Ectoedemia* species with parthenogenetic reproduction (Bond & van Nieukerken, 1987). As far as known the species is diploid (see discussion), occurs in the absence of closely related bisexuals, and is not a hybrid. Male moths have been encountered sporadically (Bond & van Nieukerken, 1987). However, the occurrence of males has been reported in a number of obligatory parthenogenetic species, usually resulting from disturbances in cell division, and is not necessarily evidence for bisexuality. The present paper is aimed at characterizing patterns of genetic variability at enzyme loci in geographic populations of *E. argyropeza* and establishing the amount of dispersal between them.

Materials and methods

Eggs of *E. argyropeza* are laid on one side of the petiole of *Populus tremula* leaves. Starting as a gallery inside the petiole, the mine develops into a blotch in the leaf lamina when the larva has reached its final instar. Under crowded conditions two mines per leaf on either side of the midrib can rarely be found.

Collection sites of *E. argyropeza* were (Fig. 1; abbreviations used in Tables in parentheses; numerals refer to year of sampling; all sampled from the Netherlands unless otherwise stated): Winterswijk-North (Wwc79); Winterswijk-East (Wwp79); Chalkney Wood (Great Britain, Sussex; Cw79); Wassenaar (Wa79); Denekamp (De80); Winterswijk-South (Wwg80); Eijs (Eij81); Orillia (Canada, Ontario; Co81); Winterswijk-East (Wwp81); Winterswijk-South (Wwg81); Bergen (Be81); Oostvoorne (Ov81); Blankenheim (Western Germany;

Fig. 1. Collection sites of *E. argyropeza* in the Netherlands and Western Germany. 1. Bergen; 2. Birresborn; 3. Blankenheim; 4. Denekamp; 5. Eijs; 6. Oostvoorne; 7. Santpoort; 8. Wassenaar; 9. Wiesbaum; 10. Winterswijk.

Bk82); Wiesbaum (Western Germany; Wi82); Birresborn (Western Germany; Bi82); Winterswijk-South (Wwg82); Bergen (Be82); Santpoort (Sa82). The term population is used throughout the text for a sample taken from either an isolated tree or various trees covering 25 m² at most.

Leaves with tenanted mines were collected in the field. Last instar larvae were removed from their mines, ectoparasitoid larvae discarded, and deep frozen at -30 °C until used for electrophoresis. Occasionally larvae were reared in the laboratory and emerging adults were stored. Larvae were individually homogenised in 60 µl distilled water. Centrifugation was found to be unnecessary. The supernatant was absorbed on Whatmann 3MM paper wicks and applied to 12% (w/v) Connaught starch gels. Electrophoresis and staining procedures followed

Menken (1982b). The following 2 buffer systems were used:

1. Running buffer: 0.223 M trizma, 0.086 M citric acid, pH 6.3; Gel buffer: 0.008 M trizma, 0.003 M citric acid, pH 6.7.
2. Running buffer: 0.03 M lithium hydroxide, 0.2 M boric acid, pH 8.1; Gel buffer: 0.002 M citric acid, 0.013 M trizma, pH 8.2.

The 6 enzymes that appeared to be polymorphic after an initial screening of 19 enzymes are listed in Table 1. All were coded for by the same gene in the larval and adult stage.

Genetic interpretation of the allelic (strictly speaking, electromorph) configurations was necessarily inferential. Therefore, the 6-locus clones actually represent enzyme phenotypes rather than genotypes. This especially pertains to non-functioning alleles which can be detected only when homozygous. In addition, some bands might originate from endoparasitoid enzymes (Saura *et al.*, 1976; Menken, 1982a; Menken & Ulenberg, 1987). Especially the absence of heterodimers in dimeric enzymes usually indicates that the band does not belong to the normal variation of the individual under study (Menken & Ulenberg, 1987). However, both bands at the *Pgi* locus of clone 23 (Table 2) migrated exactly the same distance as B and C, alleles normally present in *E. argyropeza* populations. Nevertheless two enzymes substantially differing in their primary amino acid sequence might take the same position on a gel due to insensitivity of the technique and chance events (e.g., Hase *et al.*, 1977). In case of parasitization aberrant patterns at more than one locus usually occur (Menken, 1982a), but were not found in clone 23. Finally, the homology of alleles B at the *Lap-1* locus

Table 2. Six-locus genotypic characterization of 34 clones encountered in *E. argyropeza*. Alleles are labelled alphabetically in increasing order of electrophoretic mobility

Clone	PGM	PGI	LAP-1	ME	MDH	NADH.DH-2
1	AA	BB	CC	BB	AA	BB
2	AA	BC	CC	BB	AA	BB
3	AB	BB	CC	BB	AA	BB
4	AC	BB	CC	BB	AA	BB
5	AC	BC	CC	BB	AA	BB
6	AC	BD	CC	BB	AA	BB
7	AC	CC	CC	BB	AA	BB
8	AC	BB	AC	BB	AA	BB
9	AC	BB	BC	BB	AA	BB
10	AC	BB	CD	BB	AA	BB
11	AC	BB	CC	AB	AA	BB
12	AC	BB	CC	BC	AA	BB
13	BB	BB	CC	BB	AA	BB
14	BB	BC	CC	BB	AA	BB
15	BB	BB	BC	BB	AA	AB
16	BB	BB	CC	BB	AA	AB
17	BC	BB	CC	BB	AA	BB
18	BC	BC	CC	BB	AA	BB
19	BC	CC	CC	BB	AA	BB
20	BD	BB	CC	BB	AA	BB
21	BD	AB	CC	BB	AA	BB
22	BD	AA	CC	BB	AA	BB
23	BD	BC*	CC	BB	AA	BB
24	CC	BB	CC	BB	AA	BB
25	CC	AB	CC	BB	AA	BB
26	CC	BB	CC	AB	AA	BB
27	CD	BB	CC	BB	AA	BB
28	CD	BC	CC	BB	AA	BB
29	CD	BB	CC	AB	AA	BB
30	CD	BB	CC	BB	AB	BB
31	CE	BB	CC	BB	AB	BB
32	DF	BB	CC	BB	AA	BB
33	DF	AB	BC	BC	AA	BB
34	DF	BB	CC	AB	AA	BB

*lacks heterodimer

Table 1. Enzyme assays of *E. argyropeza*. ¹See text for composition of buffers symbolised. ²M, monomer; D, dimer; X, multimer (heterozygote exhibits an activity intermediate between the two homozygotes, separate bands not resolved)

Enzyme system (E.C.Number)	Locus abbreviation	Buffer system ¹	Subunit composition ²	Number of alleles
Malate dehydrogenase (1.1.1.37)	<i>Mdh</i>	I	D	2
Malic enzyme (1.1.1.40)	<i>Me</i>	I	X	3
NADH dehydrogenase (1.6.99.3)	<i>Nadh.dh-2</i>	II	X	2
Leucine aminopeptidase (3.4.11.1)	<i>Lap-1</i>	II	M	4
Phosphoglucose isomerase (5.3.1.9)	<i>Pgi</i>	I, II	D	5
Phosphoglucosomutase (5.4.2.2)	<i>Pgm</i>	I	M	6

and alleles A and C at the *Me* locus were difficult to assess due to unresolved bands and the absence of side-by-side comparisons.

Genotypic (clonal) diversity within populations was measured according to the formula for average heterozygosity $H_g = 1 - \sum p_i^2$, where p_i is the frequency of the i th genotype (clone). The amount of genetic differentiation between any 2 populations was calculated according to Hedrick's probability of genotypic identity (Hedrick, 1971),

$$I_{x,y} = \frac{\sum_{i=1}^n p_{i,x} p_{i,y}}{1 - \left(\sum_{i=1}^n p_{i,x}^2 + \sum_{i=1}^n p_{i,y}^2 \right)}$$

where $p_{i,x}$ and $p_{i,y}$ are the frequencies of the i th genotype (clone) in population x and y and n is the number of clones.

Results

Geographic variation

A total of 32 clones were observed in the 18 population samples given in the Materials and methods section. Their genotypic composition at 6 variable loci is given in Table 2 (also including clones 26 and 34; see below). Clonal composition and frequencies in the populations studied are given in Table 3. The number of clones per population ranged from 1 to 10 with a mean ($\pm$ s.d.) of 4.7 ± 2.3 . Sixty percent of the clones (with a mean frequency of 0.032 ± 0.030) were restricted to single populations, and the mean number of populations containing a particular clone was 2.6. The latter figure increased to 4.9 if clones that occurred only once were excluded.

Clone 4 was predominant (frequency over 80%) in about half the populations. It appeared to be absent from all populations in the western part of the Netherlands (except for Bergen) and the sample from Great Britain. If clone 4 was rare or absent clones 21 and/or 27 formed the main body of the variation in a population. The 3 populations from Western Germany formed exceptions to this rule,

consisting of clone 1 (specific for this group of populations) and to a lesser extent clone 13, besides a series of rare clones (Table 3). Just over 50% of the 812 individuals electrophoresed belonged to clone 4 and 25.6% to clone 27. The other 30 clones were found at frequencies less than 5%. Twenty of them were unique to one population, 11 were represented by one individual only. All 34 clones could be derived from at least one other clone by a single mutational step (strictly speaking the difference of one electromorph), except for clone 33 which was 3 steps away from any clone encountered in this study. Theoretically possible relationships among clones are depicted in Fig. 2. Clones 1, 13 and 24 were totally homozygous genotypes; clones 1 and 24 were one mutation away from clone 4.

Genotypic diversity ranged widely from 0.000 to 0.779, with a mean of 0.352 ± 0.273 (Table 3). Finding a clone is partly a function of varying sample sizes (see the detailed analysis of Hebert *et al.*, 1988). Sample sizes differed over one order of magnitude (Table 3). Although the smallest sample (Ov81) was monomorphic for clone 21 and the largest sample (Wwg81) exhibited the largest number of clones the correlation between sample size and clonal variability as measured by the number of clones encountered was low ($r = 0.347$), and in fact a low negative correlation was observed ($r = -0.365$) if genotypic diversity was used as the measure of clonal variability. This indicated that samples reflected the real diversity.

Pgm is by far the most variable locus; with 10 genotypes and 6 alleles it explained 90.8% of the total diversity. *Pgi*, made up of 7 genotypes and 5 alleles, explained another 6.7%. The remaining 4 loci thus contributed very little to the overall clonal variability.

The ubiquity of clone 4 resulted in a bimodal distribution of Hedrick's (Hedrick, 1971) genotypic identity between populations (Table 4). Either $I_{x,y}$ values are high (if populations are compared with clone 4 predominant) or low/zero (if only one population is of the clone 4 type or if both populations lack this clone, e.g., Be81 and Ov81). To put it differently, populations sharing between 30 and 70% of their clonal diversity are rare; they comprise about 8% of the comparisons.

Table 3. Clonal composition and frequencies of *E. argyropeza* populations. Also indicated are number of specimens investigated (N_i ; all larvae except for 27 adults in Wwp79), number of clones found (N_c), and clonal diversity (H_g)

Code	N_i	N_c	H_g	CLONES														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Wwc79	27	8	0.774				0.074											
Wwp79	62	4	0.153				0.919						0.016			0.048		
Cw79	31	6	0.741													0.097		
Wa79	27	2	0.444															
De80	21	4	0.332				0.809	0.048								0.048		
Wwg80	80	4	0.118				0.938		0.013									
Eij81	20	4	0.625				0.450											
Co81	30	2	0.064				0.967				0.033							
Wwp81	26	3	0.145				0.923									0.038		
Wwg81	140	10	0.307				0.829					0.014		0.007	0.014	0.021		0.007
Be81	125	4	0.078															
Ov81	10	1	0.000															
Bk82	27	6	0.507	0.667	0.037	0.037										0.185	0.074	
Wi82	22	7	0.703	0.500		0.091	0.091									0.091		
Bi82	23	7	0.779	0.304			0.130									0.304		
Wwg82	64	5	0.204				0.890									0.016		
Be82	53	3	0.074															
Sa82	24	4	0.296				0.833	0.042		0.083								
CLONES																		
16	17	18	19	20	21	22	23	24	25	27	28	29	30	31	32	33		
0.111	0.037			0.296				0.074		0.037					0.333	0.037		
	0.193				0.322	0.065				0.323					0.016			
					0.333					0.667								
				0.095														
	0.012				0.037													
				0.100				0.400		0.050								
	0.064			0.007	0.029		0.007									0.038		
						1.000												
										0.074								
	0.136								0.045	0.045								
	0.043			0.087					0.043	0.087								
	0.031	0.047	0.016															
						0.042												

Populations that were sampled from approximately the same locality in different years yielded I_{x-y} values of 0.990 or higher (Wwp79-81, Wwg80-81-82, Be81-82). The last case comprised an isolated tree; the very small temporal changes in clonal composition could be fully explained by sampling error.

Fine scale population structure

A detailed analysis of local population structure was undertaken close to the place where populations Wwp79 and Wwp81 were sampled. During 5 consecutive years 4 individual trees were studied (Ta-

were found, both only once.

The following comparison is restricted to trees 1 and 2. Despite their close vicinity clonal composition differed significantly between the 2 trees (Table 6). Some clones consistently occurred on one tree while being absent from the other, viz., clone 12 on tree 2 and clones 20 (with one exception in 1981) and 32 on tree 1, neglecting clones that occurred only once. Total population sizes for tree 1 and 2 for the first two years of sampling were estimated at 300 and 700 respectively. Almost all leaves on tree 2 were tenanted, occasionally with 2 mines in one leaf (see Materials and methods section). In 1983 population size on tree 1 dropped to some 100 and the next year only 2 tenanted plus a few vacated mines were found. In 1985 no mine was observed. Population sizes on tree 2 gradually declined to some 300. Thus there was increasing opportunity for genetic drift to operate.

Intensive sampling in such small populations undoubtedly will influence the composition of the next generation. For instance, clone 20 was the second most common clone on tree 1 in 3 consecutive years. It was once observed on tree 2 but apparently this individual was unable to produce progeny. Nonetheless this indicated that it was extremely rare on tree 2. Moreover, total diversity (number of clones) decreased over years on all trees (Table 5). At any rate sampling error alone cannot account for the rar-

ity or absence of clone 20 on tree 2 ($P < 0.005$ for 1981 and $P \leq 0.001$ for 1982 and 1983; Table 6). Neglecting sampling effects the population composition did not change much temporally, corroborating results mentioned above.

Rare clones can be used to detect dispersal in a way similar to the private allele method described by Slatkin (1985). Average frequencies of private clones (uncorrected sample size) were 0.040, 0.098 and 0.150 for the first 3 years of sampling. The large differences can partly be explained by the fact that in 1981 clone 20 was not private, and the absence of quite a few single individual clones in 1983 (due to sampling). An average frequency of 0.100 indicates an average exchange of less than 1 individual per generation between stands (compare Table 7 in Slatkin, 1985), the more so because really nearby locations were sampled. Such a concentrated sampling would result in a higher estimate of dispersal than when more remote populations are studied (Slatkin, 1985; Menken, in press).

Discussion

All data corroborate that *E. argyropeza* lacks sexuality. It possesses a clonal population structure in which recombination and genetic segregation are rare or absent. All populations deviate from Hardy-Weinberg proportions and have large linkage disequilibria (Ov81 for example), typical for clonally structured populations. Random combination of allozymes at each of the 6 loci would certainly not lead to the observation of repeatedly identical genotypes, or genotypes differing only one allele from each other.

Offspring analysis (not shown) and analysis of genetic relationships among clones in *E. argyropeza* populations suggested an apomictic mode of parthenogenesis. Yet cytogenetic analysis showed meiosis to occur (L. Pijnacker, pers. comm.). *E. argyropeza* probably displays a mode of parthenogenesis similar to the one found in the stick insect *Carausius morosus* Br. (Pijnacker & Ferwerda, 1986). In *Carausius* meiosis is coupled with apomixis. Chromosomes double prior to meiosis but pairing is restricted to sister chromosomes. Crossing overs in such

Table 6. Probability (P) according to the binomial distribution (see Menken, 1981) of failing to find a clone or finding one (clone 20, 1981) clone on the other tree given sample size (n) and assuming observed frequency (q) is the same on both trees

Year	n	q	P
Clone 12			
1981	45	0.075	0.030
1982	48	0.032	0.210
Clone 20			
1981	53	0.133	<0.005
1982	62	0.167	≤ 0.001
1983	52	0.210	≤ 0.001
Clone 32			
1981	53	0.044	0.092
1982	62	0.042	0.070
1983	52	0.091	0.007

autobivalents have no genetic consequences, leading to offspring genotypically identical to the mother.

The diploid character of *E. argyropeza* has been deduced from the chromosome number (n = approximately 30) and electrophoretic patterns. None of the specimens examined appears to carry more than 2 alleles at any locus. This is not necessarily proof of diploidy though, as Lokki *et al.* (1975) observed only di-allelic plus a few tri-allelic genotypes among tetraploid populations of *Solenobia triquetrella* Hübner. This may be explained by the short time since origin of the parthenogenetic form (Lokki *et al.*, 1975) and what Sugai and Mirumachi (1973) refer to as mutation resistance, or competitive inferiority of the polyploids (see below).

E. argyropeza harbours substantial numbers of clones: electrophoresis revealed a total of 34 clones with an average of 4.7 clones per population. Polyclonal species vary much in the extent of diversity (e.g., Lokki *et al.*, 1976 and references therein; Parker *et al.*, 1977; Selander *et al.*, 1978; Mitter *et al.*, 1987). This can be explained in part by differences in sampling and sample size (Hebert *et al.*, 1988), and number of loci on which a clonal genotype is based. Furthermore, differences in clonal diversity might reflect differences in their mode of transition to asexuality, in time since this transition (assuming a molecular clock), and in ecological and population genetical factors (such as bottlenecks, migration, drift, and replacement of less-adapted genotypes). However, the relative contributions of these factors are difficult to assess due to lack of power of quantitative analytical methods (Hebert *et al.*, 1988).

Analysis of geographic variation patterns in *E. argyropeza* revealed appreciable differentiation, even over short distances. Low dispersal rates together with chance events in small (sub)populations or differences among clones in their capacity for survival could explain the observed clonal distribution. The absence, for instance, of clone 4 from 3 of 4 populations in the western part of the Netherlands (and the single sample from England) can be partly explained by genetic drift. The predominance among our samples of clone 4 namely might result from the overrepresentation of populations around Winterswijk in the eastern part of the Netherlands, where clone 4 dominates. Yet populations Wwc79

and Wwp79, only a few kilometers apart, exhibit a genotypic identity of only 0.137 as clone 4 is almost lacking from Wwc79. Instead this population largely consists of clones 20 and 32, which are both nearly absent from the Winterswijk area (Table 3).

The Bergen population (Be81 and Be82) probably founded after a maximum possible bottleneck (a single female belonging to clone 27) constitutes another example of drift. The rare clones 28, 29, 30, and 31 most likely originated from the ancestral clone 27 after the introduction through single mutations (Table 2; Fig. 2); they are unique to this population. In 1983 no mine, tenanted or vacated, could be found on the isolated tree that sustained the Bergen population. Depending on the size and composition of a new introduction the genetic make-up of a future population could be very similar, or totally different from the Be81 and 82 ones. The case of the Bergen population implies that large fluctuations in population size, a phenomenon not uncommon in insects, occur in *E. argyropeza*. Thus genetic drift is an important factor that moulds the clonal variability. As a consequence nearby populations can be highly differentiated and distant populations similar by chance, although the reverse situation is expected to be more common (Wright, 1943).

Fig. 2. Interrelationships among clones in *E. argyropeza*. Lines indicate that 2 clones differ at one allele from each other. Small digits refer to clones unique to a population.

E. argyropeza downesi, a subspecies feeding on *Populus tremuloides*, has been described from North America by Wilkinson and Scoble (1979). Genetic analysis, however, makes a recent introduction through a severe bottleneck much more likely. Probably a single female with clone 4 genotype founded the new population (Co81; Table 3). The only deviant genotype (one individual of clone 8) has not been found in other samples and could be derived from clone 4 through a single mutation at the *Lap* locus. Thus a subspecific status is not warranted (see also van Nieukerken, 1985). The above mentioned phenomenon at the *Lap* locus holds for the majority of genotypes that are unique to a population; 65% can be derived directly from the (second) most common clone in that population (Table 3). Such a high incidence of new clones, however, requires a very high mutation rate. Alternative explanations include the occurrence of some crossing over and complex scenarios of dispersal, selection, and local extinction. The complex theoretical interrelationships in Fig. 2 might suggest crossing over, but -apart from problems in homology assessment- our knowledge is too fragmentary to draw firm conclusions.

At any rate, high predictability of the host plant *P. tremula* should not select for an insect with good dispersal (Southwood, 1962; Dingle, 1972; Menken, in press). In particular the analysis of the fine population structure renders it likely that *E. argyropeza* has a highly localized structure. Females which pupate in the soil underneath the tree from which they have fed as a larva most likely deposit their eggs on that very tree. Most oviposition is within 24 h after eclosion. Direct observations show that they rarely fly, but instead run over leaves and hop from leaf to leaf. Active dispersal thus seems to be unimportant; (re)colonization of isolated stands may be occurring via passive dispersal by wind. In conclusion, available information hints at low dispersal as the primary cause of differentiation within a population, and perhaps, among populations.

Even if dispersal is occurring during their oviposition period the total collection of host plants will be a fine-grained environment (Levins, 1968); if selection maintains the diversity then overdominance can maintain polymorphisms (Strobeck, 1975), a condi-

tion clearly not feasible for apomictic organisms. Only some form of diversifying selection might explain the clonal diversity. Environmental heterogeneity in the form of phenological variation among food plants (Edmunds & Alstad, 1978; Mitter *et al.*, 1979) and density-dependent selection (Futuyma *et al.*, 1981) have been suggested. There is accumulating evidence that even coexisting clones are not always ecologically equivalent and do not necessarily represent general purpose genotypes with high mean fitness (Lynch, 1984). Rather clones often have narrow ecological preferences (Scudday, 1973; Mitter *et al.*, 1979; Vrijenhoek, 1979; Young, 1983; Harshman & Futuyma, 1985). However, since ecological differences are not always discerned (Jaenike *et al.*, 1980), some clonal variation might be selectively neutral (Parker, 1979).

Although we do not have information on differences in phenology among geographic populations of *P. tremula* it is unlikely that such differences occur within small stands. New trees of *P. tremula* can sprout from roots at least 40 m away from the main trunk. Probably this situation exists in the population in which the fine structure was studied (Table 5).

Fitness differences arise more rapidly among asexual lineages that originate repeatedly (polyphyletically) than among those that are monophyletically produced by single mutations from another clone as in *E. argyropeza* (Maynard Smith, 1978; Harshman & Futuyma, 1985). It should be kept in mind that the genome of *E. argyropeza* is inherited and thus selected as a unit, such that changes at allozyme loci often represent passive consequences of selection at other loci.

Moreover, it should be clear that only a fraction of the total clonal diversity is discerned in this study. If specimens from natural populations are used, genotyping is necessarily weak. Apart from the well-known problem of heterogeneity within electrophores (King & Ohta, 1975) a clone is solely characterised by 6 loci as markers of the entire genome. A given clone possibly comprises a heterogeneous collection of genotypes with an identical 6-locus electrophoretic profile. Carried to extremes (total DNA sequence) most if not all offspring of even an apomictic female would differ due to mutations, but

a large fraction of the mutations will have infinitesimally small effects on fitness. Any insight in the infrastructure of a clone with respect to variation in dispersal abilities, competitiveness etc. is lacking. This especially holds true of the abundant and widespread clone 4. Clones unique to a population on the other hand are far less likely to be genetically heterogeneous. For this reason they are good population genetic markers. Thus rare clones indicate sedentary behaviour (compare Slatkin, 1985), unless such local clones have lowered fitness. The near absence of clone 20 from tree 2 (Table 5), for instance, can hardly be explained by assuming lowered fitness on this tree as the host trees are probably physiologically (almost) identical. This view is supported by the occurrence of clone 20 on tree 2 in 1981 which indicates that it can survive at least until the last instar. Another possibility is that clone 20 has an inherently low dispersal ability.

E. argyropeza clearly represents a monophyletic lineage despite the large number of clones encountered. The species is genetically fairly monomorphic. Over half of the parthenogenetic individuals are genetically identical (clone 4), and with one exception the remaining clones can be derived from this clone by single mutational steps that have taken place after the origin of parthenogenesis (Fig. 2; Suomalainen & Saura, 1973; Lokki *et al.*, 1975). Species with a supposed polyphyletic origin are much more diverse and the clones that make up their populations typically differ from one another by at least 2 mutations (Lokki *et al.*, 1975; Mitter *et al.*, 1979).

Over 90% of the overall variability could be ascribed to variation at the *Pgm* locus. Heterozygote genotypes greatly outnumber homozygote genotypes at this locus (7 versus 3; 90 versus 10% of the individuals). This is a conservative estimate as, for instance, a BB phenotype is more likely to be heterozygous for B and a null allele than that it is a real BB homozygote. This results from the apomictic mode of reproduction, where mutations can only become homozygous in the rare case of a new identical mutation, unless BB represents the ancestral situation. Non-functional alleles may be selected against as heterozygotes (Pamilo, 1987). If so their frequency is kept low, whereas functional heterozygosity increases (Pamilo, 1987), as observed for *Pgm*. The

large amount of variation at the *Pgm* locus was expected as it is the most variable locus among some 30 bisexual species of *Ectoedemia* (Menken, in prep.), with a mean heterozygosity of 0.443 ± 0.223 (range 0.000–0.814).

The amount of heterozygosity in a parthenogenetic diploid depends on initial heterozygosity (in non-hybrid species similar to that of related bisexual species, in species originating from hybridization between bisexual species an order of magnitude greater than that of related bisexuals), mutation rate and time since origin (Lokki, 1976). As for the ancestral species *E. klimeschi* (Skala) is the most likely candidate (van Nieuwerkerken, 1985). Unfortunately only one *E. klimeschi* population has been investigated electrophoretically. Its heterozygosity level and pattern of genetic variation were similar to that in *E. argyropeza*, corroborating other studies pertaining to parthenogenetic lineages and their bisexual ancestors (Suomalainen *et al.*, 1976). Species comparison revealed that out of 16 genetic loci, 7 were identically monomorphic (e.g., *Mdh*), 5 more loci overlapped largely in their allozyme contents (e.g., *Pgi*, *Lap-I*, *Me*, and *Nadh.dh-2*) and 3 were diagnostic. The situation for the last locus (*Pgm*) depends on the *E. argyropeza* population to which *E. klimeschi* is compared since *E. klimeschi* is heterozygous for alleles C and D (with frequencies of 0.600 and 0.300, respectively) besides two low frequency alleles not occurring in *E. argyropeza*. This means that the present study might include *E. klimeschi* individuals with a genotype identical to clone 27 were it not that *E. klimeschi* never feeds on *P. tremula* but only on *P. alba*. The corresponding genetic identity according to Nei (1972) between *E. argyropeza* and *klimeschi* amounts to 0.810 based on 16 genetic loci. If *E. klimeschi* really constitutes the ancestral bisexual species it means that clone 27 is the most primitive one in *E. argyropeza*. It is the second most common clone in *E. argyropeza* and separated by only one mutation (D→A at the *Pgm* locus) from the most common clone (clone 4).

The common occurrence of parthenogenetic taxa in various insect orders demonstrates their success. They are quite well capable of adaptive evolution. Nonetheless, the rapid accumulation of harmful versus the slow accumulation of beneficial mutations

may be important factors why parthenogenetic forms are outcompeted in the long run by taxa with bisexual reproduction (Maynard Smith, 1978; Templeton, 1982; Lynch, 1984; Pamilo *et al.*, 1987).

The present study suggests that populations of *E. argyropeza* are highly sedentary. Small population sizes and bottlenecks together with extinction and passive recolonisation most likely explain the observed diversity patterns. Studies of population structures in other, sexually reproducing, Nepticulidae are in progress.

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