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Genetic polymorphism and evolution in parthenogenetic animals

V. Triploid *Adoxus obscurus* (Coleoptera: Chrysomelidae)

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SUMMARY

The genetic variability at 16 enzyme loci in 52 Scandinavian triploid parthenogenetic populations of the beetle *Adoxus obscurus* has been studied by starch-gel electrophoresis. The overall genotypes of different beetles have been compared with each other and with a sample from a diploid bisexual population from Canada. Eighty per cent of the parthenogenetic beetles have the same overall genotype. The remainder belong to six genotypes, three of which are found in only one population. The variability within and between parthenogenetic populations of *A. obscurus* is much lower than in other comparable parthenogenetic insects studied by us. This is interpreted to be a consequence of the efficient migration of the genotypes with the highest fitness, leading to a replacement of less-adapted genotypes. *A. obscurus* is a flying insect, whereas the other parthenogenetic insects studied by us are sluggish flightless forms.

1. INTRODUCTION

Adoxus obscurus L. is the only species of the genus *Adoxus*. It has a very extensive distribution. According to Horion (1951, p. 399) this beetle is spread over Europe and Siberia all the way to Japan; in addition it occurs in North America. In California it is a pest of grape and has the common name western grape rootworm. Jobert (1881) and Jolicoer & Topsent (1892, according to Székessy, 1937) were the first to point out that *Adoxus obscurus* reproduces parthenogenetically. Jobert reports that he did not find a single male among 3728 individuals studied. He also found no sperm in the receptacula seminis of the females, even though the eggs developed normally. Likewise, Weise (1898) studied very many individuals without noticing a single male. In the beginning of this century the parthenogenesis of *Adoxus obscurus* fell into doubt, since various authors (cf. Müller, 1904; Schaufuss, 1916, p. 925; Winkler, 1920, p. 73) reported that males were found in central and southern Europe. According to Vandel (1931, p. 198), Balbiani had found that three out of six – presumably Italian – individuals were males. Likewise, Müller (1904) encountered a single male near the town of Graz

in Austria (this individual was certainly a male, since Müller's description is accompanied with a drawing of the genitalia, which include normal-looking testes). According to numerous later authors (e.g. Vandel 1931; Székessy, 1937; Lindroth, 1943) only females are found in many localities in Europe. Cytological studies (cf. below) have confirmed the parthenogenesis of *Adoxus obscurus*. The possible bisexuality in certain localities in southern and central Europe is, however, not excluded. The occurrence of single males is not necessarily evidence for bisexuality. Single males have been found in numerous obligatory parthenogenetic species. These males have resulted from disturbances in cell divisions.

Within its northern American area of occurrence *Adoxus obscurus* is bisexual. Parthenogenesis has not been reported from North America.

Cytological studies (Suomalainen, 1965) showed that the parthenogenetic populations from Europe (at least from Finland and Switzerland) are triploid with a chromosome number of 24. In the bisexual race $2n = 16$. The parthenogenesis is of the apomictic type. There is no chromosomal conjugation and only a single equational maturation division occurs in the formation of the eggs. The chromosome number 24 is observed also in the cleavage divisions.

We have previously (Suomalainen & Saura, 1973; Lokki *et al.* 1975; Saura *et al.* 1976*a*, *b*) studied the genetic polymorphism and evolution of parthenogenetic insects. Each different parthenogenetic genotype was found to have a peculiar geographic distribution. These distributions reflect the evolution of genotypes from each other through mutations. This was due to the circumstance that all species studied by us (weevils, bagworm moths) were flightless and had a very limited power of active dispersal. *Adoxus obscurus* is an actively flying beetle. Studying the genetic polymorphism of parthenogenetic animals having different mobilities may give information on the interaction between mutation and selection in the absence of recombination. In this study we describe the genetic polymorphism in the populations of a flying parthenogenetic insect, *Adoxus obscurus*.

2. MATERIALS AND METHODS

(i) *Experimental animals*

Thirty-one diploid bisexual *Adoxus obscurus* beetles were collected at Bells Corners, Ontario, Canada (45° 19′ N, 75° 54′ W) by Dr Edward C. Becker in July 1974. They were sent live to our laboratory and analysed with triploids as controls. Triploid beetles were collected at 52 localities in Scandinavia (Fig. 1), by sweeping fireweed (*Chamaenerion angustifolium*) with a net. In general, four individuals were assayed from each locality. In order to establish the variability within a population, over 30 beetles were assayed from two localities.

Table 1 gives for each sample the locality indicator (serial number), the collection year and the number of individual beetles assayed. The last column gives, for the samples collected in Finland (numbers 1–37), the number in the grid catalogue for biological finds in Finland (Heikinheimo & Raatikainen, 1971). The Swedish samples (38–52) are designated by coordinates of the locality.

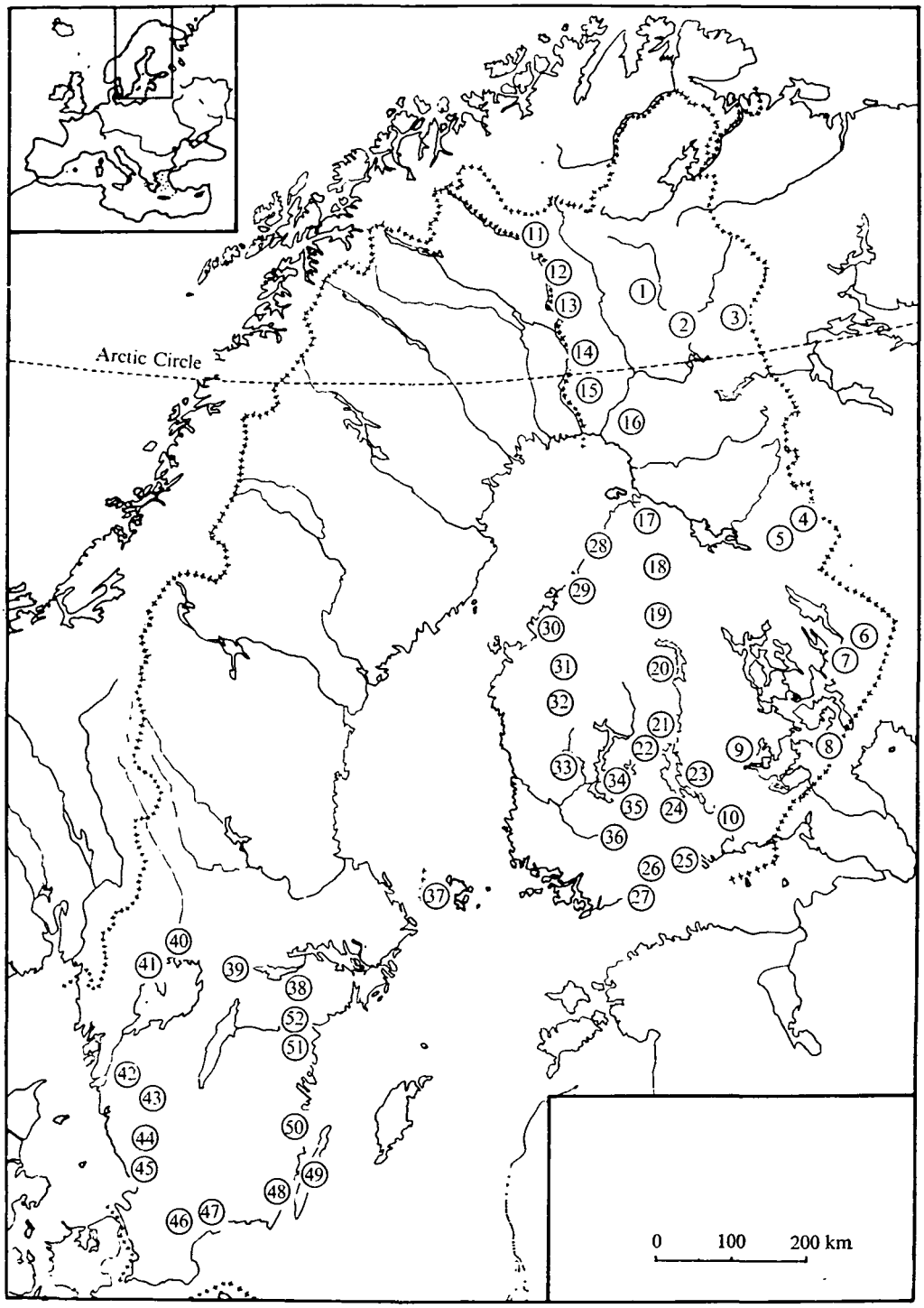

Fig. 1. The collection localities of parthenogenetic *Adoxus obscurus*.

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Table 1. *The collecting localities of the* Adoxus obscurus *samples studied* (*Consult text for details*).

Locality indicator	Year	No. of individuals assayed	Code of locality	Locality indicator	Year	No. of individuals assayed	Code of locality
1	75	4	748:46	26	73	2	668:37
2*a*	74	5	744:52	27	75	4	666:36
2*b*	75	4	744:51	28	75	4	714:36
3	74	5	741:57	29	75	4	709:32
4	74	4	714:63	30	75	4	704:27
5	73	33	713:59	31	75	4	698:29
6	74	4	697:68	32	75	4	693:28
7	73	36	694:65	33	75	4	685:28
8a	75	9	683:63	34	74	4	681:34
8b	75	14	682:62	35a	74	4	678:35
9	75	4	682:51	35b	75	18	678:35
10	74	20	675:48	36	74	4	674:33
11	75	4	757:34	37	75	4	669:09
12	75	4	754:36	38	75	5	59° 05′ N 16° 26′ E
13	75	4	748:35	39	75	4	59° 17′ N 14° 26′ E
14	75	4	741:36	40	75	1	59° 25′ N 13° 33′ E
15	74	5	737:40	41	75	6	59° 16′ N 12° 59′ E
16	74	5	730:42	42	75	4	57° 54′ N 12° 33′ E
17	74	5	718:43	43	75	2	57° 41′ N 13° 04′ E
18	74	5	710:43	44	75	2	57° 02′ N 12° 57′ E
19	74	5	704:43	45	75	4	56° 47′ N 12° 57′ E
20	74	5	696:43	46	75	1	56° 06′ N 13° 55′ E
21	74	5	687:41	47	75	4	56° 07′ N 14° 40′ E
22	74	5	685:38	48	75	6	56° 30′ N 16° 07′ E
23	75	4	681:44	49	75	5	56° 49′ N 16° 41′ E
24	74	4	676:40	50	75	4	57° 17′ N 16° 27′ E
25*a*	73	1	669:41	51	75	4	58° 18′ N 16° 31′ E
25*b*	74	4	669:41	52	75	2	58° 41′ N 16° 24′ E

(ii) *Laboratory procedures*

Live beetles were rapidly frozen, and stored in a deep freeze for about 2 weeks. They were then homogenized in a glass and lucite plastic tissue homogenizer and the samples were electrophoresed in a starch gel as described by Suomalainen & Saura (1973). Controls were placed in the gels at intervals of eight samples.

The following enzymes were assayed separately for each weevil: acid phosphatase (Acph), adenylate kinase (Adk), amylase (Amy), Esterase (Est), hexokinase (Hk), isocitrate dehydrogenase (Idh), leucine aminopeptidase (Lap), malate dehydrogenase (Mdh), malic enzyme (Me), superoxide dismutase (Su) and triosephosphate isomerase (Tpi). The staining procedures are in general use (e.g. Suomalainen & Saura 1973).

3. RESULTS

Enzyme loci are designated with an abbreviation of the trivial name of the enzyme. When several loci are coding for an enzyme, each locus is identified with a hyphenated number according to increasing anodal migration. Allozymes and the corresponding alleles are identified with indices showing their differences in migration from an arbitrarily chosen control allozyme.

(i) *Variability in a diploid bisexual population*

Table 2 shows the variability at nine polymorphic loci in a diploid bisexual population of *A. obscurus*. Seven other loci were monomorphic in this population and homozygous for allele *1·00*. They are: *Adk-2*, *Amy*, *Mdh-1*, *Mdh-2*, *Me*, *Su-1* and *Tpi*. The number of individuals studied is 31. The observed degrees of heterozygosity agree well with those expected on the assumption of Hardy–Weinberg equilibrium.

(ii) *Variability in triploid parthenogenetic beetles*

The following loci were consistently monomorphic in all triploid individuals studies: *Adk-2* (allele *1·00*), *Amy* (allele *1·00*), *Est*-2 (allele *1·03*), *Lap-2* (permanently heterozygous for the allele combination *1·00/1·04*), *Mdh-1* (allele *1·00*), *Mdh-2* (permanently heterozygous for *0·93/1·00*), *Su-1* (allele *1·00*) *Su-2* (allele *1·00*) and *Tpi* (allele *1·00*). In addition to these loci, *Adk-1* was monomorphic for the allele *1·00* in the material collected in 1975. This locus was not studied for the diploids or for the triploid samples collected in 1973 and 1974. Triploid parthenogenetic beetles do not show any Est-1 activity and there is no evidence that Est–1 bands would appear elsewhere in gels stained for esterases.

Fig. 2 shows the variation at loci which are polymorphic in the triploid parthenogenetic beetles. The data for monomorphic loci should be added to the overall genotype given for each beetle. When inspecting Fig. 2, it should be kept in mind that the genotype allele configurations need not be absolute. Since there is no recombination, they cannot be verified in any way. Therefore, they actually represent enzyme phenotypes rather than genotypes. This is particularly true for

Table 2. *Allele frequencies of polymorphic loci in a diploid bisexual population (Bells Corners, Ontario, Canada) of* Adoxus obscurus

(Numbers in parenthesis give the number of individuals assayed. Numbers in italics denotes alleles.)

Locus						Proportion of herto-zygotes	
						Observed	Expected
Acph-2 (31)		*0·97* 0·16		*1·00* 0·83	*1·03* 0·02	0·226	0·297
Est-1 (29)		*0·96* 0·09		*1·00* 0·71	*1·04* 0·21	0·414	0·450
Est-2 (31)		*0·97* 0·03		*1·00* 0·89	*1·03* 0·08	0·161	0·206
Est-3 (31)		*0·98* 0·11		*1·00* 0·77	*1·02* 0·11	0·323	0·375
Est-4 (31)			*1·00* 0·92	*1·02* 0·08		0·161	0·148
Idh (31)	*0·95* 0·39		*0·97* 0·07	*1·00* 0·52	*1·05* 0·02	0·677	0·562
Lap-1 (30)			*1·00* 0·67	*1·03* 0·33		0·400	0·444
Lap-2 (31)		*0·96* 0·02		*1·00* 0·81	*1·04* 0·18	0·290	0·318
Su-2 (31)			*1·00* 0·95	*1·07* 0·05		0·097	0·092

the *Est-4* genotype designated with a zero. Individuals having this genotype do not show any Est-4 activity. Est-4 stains strongly in other beetles, so that the zero phenotype may well be due to, for example, homozygosity for a 'null' allele. We have no reliable way of showing whether the genotype designated with *Est-4*$^{1\cdot00}$ is homozygous for *Est-4*$^{1\cdot00}$ or whether it contains one or two doses of the postulated null allele.

The genotypes are each identified with a Roman number. They are collectively

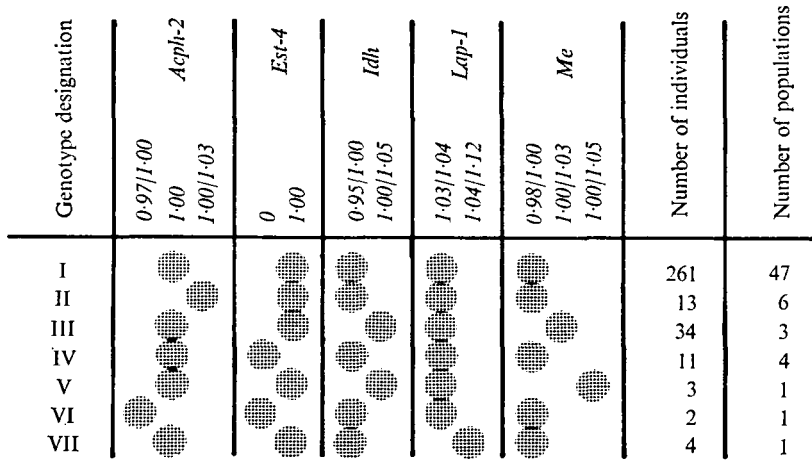

Fig. 2. A compilation of *Adoxus obscurus* genotypes. The symbol of the genotype, the overall genotype with regard to variable loci and the numbers of individuals having the overall genotype as well as the number of populations, in which the genotype has been found are given.

Table 3. *The distribution of genotypes*

	Genotype						
Population	I	II	III	IV	V	VI	VII
4	—	—	1	—	3	—	—
5*	4	—	29	—	—	—	—
6	—	—	4	—	—	—	—
8b	6	—	—	6	—	2	—
10	18	—	—	2	—	—	—
25b	3	—	—	1	—	—	—
27	2	—	—	2	—	—	—
35b	16	2	—	—	—	—	—
38	—	—	—	—	—	—	4
39	3	1	—	—	—	—	—
41	4	2	—	—	—	—	—
46–47	—	5	—	—	—	—	—
48	3	3	—	—	—	—	—

All other populations contained only genotype I: i.e. populations 1–3 (18 individuals), 7* (36), 8a (9), 9 (4), 11–24 (64), 25a* (1), 26* (2), 28–35a (32), 36–37 (8), 40 (1), 42–45 (12) and 49–52 (15). Idh and Lap-1 were not tested for populations marked *. The numbers give the number of individuals belonging to each genotype.

presented in Fig. 2. The material collected in 1973 lacks *Idh* and *Lap* data. We have combined these data (5, 7, 25a and 26) with the genotype to which they most probably belong. In comparison with the widespread type I, Type II is characterized by an additional allele $Acph\text{-}2^{1.03}$. Type III has two characteristic allele combinations, $Idh^{1.00/1.05}$ and $Me^{1.00/1.03}$. Type IV has the presumed $Est\text{-}4^{0}$ in a homozygous condition.

(iii) *The geographic distribution of different genotypes*

The geographic distribution of different genotypes is presented in Table 3, and shows that, with the exception of five localities numbered 4, 6, 38, 46 and 47, Type I occurs throughout the area studied. Altogether, 261 of the 328 beetles assayed (i.e. 80 %) belong to this type. Type II has been found in 13 individuals, which originate from six populations. Five of these localities (39, 41, 46, 47 and 48) are in Sweden and one (35) in Finland. Type III occurs in 34 individuals but only in three localities (4, 5 and 6). These localities are in eastern central Finland. Type IV has been encountered in 11 individuals, which originate from four populations (8b, 10, 25 and 27). These localities are the ones from southeastern Finland. Only nine individuals represent allele combinations which do not belong to the four types mentioned above.

4. DISCUSSION

Adoxus obscurus lives on two host plant genera, fireweed (*Chamaenerion*) and grape (*Vitis*). All our material was collected from fireweed. These two plant genera have diverged into more species in the Far East and in North America than in Europe, and they may both be of American origin. Thus the American bisexual race of *A. obscurus* may well be ancestral to the Eurasian parthenogenetic one, and may have spread to Eurasia along with the food plants.

The allele constitution of the parthenogenetic race is similar to that in the single diploid bisexual population sampled, but the diploid sample was too small to allow generalizations. Thus the additional alleles observed in the triploid parthenogenetic beetles may also occur in some bisexual populations. Considering the vast geographic distance separating the North American diploid sample from the Scandinavian parthenogenetic forms, it is surprising that they resemble each other so much.

The main allele differences between the parthenogenetic and bisexual races are at four loci: *Est-1*, *Lap-1*, *Mdh-2* and *Me*. Est-1 does not stain in parthenogenetic samples, even when they are run in the same gels with bisexual samples. The parthogenetic individuals were heterozygous at *Lap-1*, *Mdh-2*, and *Me*. In addition to an allele present in the bisexual beetles they have another allele. These additional alleles characterize parthenogenetic beetles.

In all parthenogenetic animals the bisexual race must be assumed to represent the ancestral form, from which the parthenogenetic races have evolved. The Scandinavian parthenogenetic *A. obscurus* samples studied represent a clearly monopyhletic lineage. Eighty per cent of the parthenogenetic beetles were genetic-

ally identical, and the remaining 20% can be derived from these by single mutations. These mutations have occurred after the origin of parthenogenesis and triploidy.

In comparison with two comparable parthenogenetic insects, *Solenobia triquetrella* (Lokki *et al.* 1975) and *Otiorrhynchus scaber* (Suomalainen & Saura, 1973; Saura *et al.* 1976*a*) parthenogenetic *A. obscurus* is genetically highly monomorphic. *S. triquetrella* populations differ widely from each other, and all the evidence indicates that they are polyphyletic. In contrast to them, *O. scaber* genotypes differ from each other very slightly, so that the differences can be explained by single mutations. More than 70 such tetraploid parthenogentic *O. scaber* genotypes have been reported from Scandinavia, among the 482 individuals studied. In spite of this extensive variability, they represent a monophyletic lineage, and compared to them the monophyly of different parthenogenetic genotypes of *A. obscurus* is obvious.

The average heterozygosity per individual per locus in the single bisexual sample of *A. obscurus* studied is 0·18 (with S.D. 0·20). Correspondingly, the number of permanently heterozygous loci in the parthenogenetic race is 5 out of 17 loci studied. The number of different genotypes observed in the parthenogenetic race of *Adoxus* is low in comparison with other parthenogenetic insects studied by us (*Solenobia*, weevils) (Suomalainen & Saura, 1973; Lokki *et al.* 1975, 1976; Saura *et al.* 1976*a*, *b*). With the exception of *Polydrosus mollis* these insects are sluggish and flightless forms.

The major food plant of Scandinavian *Adoxus*, the fireweed, is a circumpolar plant species. It is a characteristic element of the secondary succession, which appears almost anywhere where the soil nitrogen content is elevated (e.g. following fires). *Adoxus* must be able to follow its vagrant host species. When we consider the distribution of the minor genotypes III and IV, it becomes apparent that the geographic distributions do not reflect migration barriers. The reasons for this phenomenon may be assumed to be adaptive differences of the genotypes (e.g. Types III and IV may well favour a more continental climate in comparison with Type I). The success of Type I can be explained by the assumption that it has superior fitness in Scandinavia. Whenever local genotypes originate by mutation they are in general not capable of competing with Type I and are therefore eliminated within this area. Population 38 is an example of local differentiation in our material. The population structure of an actively migrating parthenogenetic insect is very different from that of the flightless weevils. The latter are divided into numerous locally adapted populations the distribution of which follows biotic zones (cf. Saura *et al.* 1976*a*). An actively flying parthenogenetic insect, like *Adoxus*, can afford the local extinction of a poorly adapted genotype as the population may be rapidly replenished from nearby populations. This is in our opinion the explanation to the low number of different *Adoxus obscurus* genotypes in northwestern Europe.

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