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Allozyme polymorphism in the parasitic hymenoptera *Diadromus pulchellus* WSM. (Ichneumonidae)

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SUMMARY

The allozyme polymorphism of *Diadromus pulchellus* has been analysed at 22 loci, the expression of one (*Est-4*) being male-limited. Six loci were found polymorphic for two (*Ldh-1*, *Ldh-2*, *Acp-2*, *Pgi* and *Sdh*) or three (*Est-3*) codominant alleles. Allele frequencies are similar in males and females at four loci in a laboratory population but there are important heterozygote deficiencies in females at three of them. The possibilities of negative heterosis or assortative mating to explain this deficiency are discussed.

1. INTRODUCTION

Allozyme polymorphism is widely spread among diploid organisms (Pasteur, 1974; Powell, 1975) but very little is known about haplodiploid species. Up to now, multilocus analyses of Hymenoptera species have shown that there is very little enzymic polymorphism (Pamilio, Vepsalainen & Rosengren, 1975; Meltcalf, Marlin & Whitt, 1975) or even no polymorphism at all (Snyder, 1974). These observations are in accord with the hypothesis that, in Hymenoptera, the genetic variability should be very slight since all loci are exposed to selection in the hemizygous condition (Askew, 1968; Suomalainen, 1962). However, Kerr (1967, 1969) and Contel & Mestriner (1974) argue that multiple alleles may still be frequent because of the possibility of widespread over-dominance.

The analysis of the biochemical polymorphism of *Diadromus pulchellus*, a species which parasitizes the Lepidoptera *Acrolepiopsis assectella* during its whole preimaginal life, shows that the last hypothesis could hold, at least in some cases: *Diadromus* was found to be polymorphic at some 30 % of its loci. Our results are presented below.

2. MATERIAL AND METHODS

Diadromus pulchellus imagos were reared in $40 \times 30 \times 24$ cm cages at a relative humidity of 70 ± 10 % and thermo- and photoperiods of 16 h day at 25 ± 1 °C and 8 h scotophase at 15 ± 1 °C. Every day, pupae of *Acrolepiopsis assectella* 24 h old

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were presented to *Diadromus* females for oviposition (Labeyrie, 1960). The host was bred on an artificial medium described by Pralavorio, Arambourg & Guennelon (1973).

Formal genetic studies were performed on the progeny of single virgin or mated (forced copulation) females, while the population study was done on imagos captured at random from a stock cage. In this cage, new field *Diadromus* were introduced every year in September, in order to avoid any consanguinity effect. These *Diadromus* were captured at Siagne (Alpes maritimes, France).

Table 1. *Enzymatic systems and corresponding loci analysed to estimate the polymorphism of* Diadromus pulchellus

Enzymes	Electrophoresis buffers	Staining procedures	Encoding loci	Monomorphism or polymorphism*
α-Glycerophosphate DH	Tris-citrate	Pasteur & de Stordeur, 1976	*α-Gpd-1* *α-Gpd-2*	M (105) M (100)
Sorbitol DH	Tris-borate	Ayala *et al.* 1972	*Sdh*	P (211)
Lactate DH	Tris-borate	Selander *et al.* 1971	*Ldh-1*† *Ldh-2*	P (124) P (189)
Malate DH	Tris-citrate	Selander *et al.* 1971	*Mdh-1* *Mdh-2*	M (87) M (127)
Malic enzyme	Tris-citrate	Selander *et al.* 1971	*Me-1* *Me-2*	M (74) M (106)
Isocitrate DH	Tris-citrate	Selander *et al.* 1971	*Idh-1* *Idh-2*	M (15) M (15)
6-Phosphogluconate DH	Tris-citrate	Ayala *et al.* 1972	*6-Pgd*	M (15)
Glutamate-oxaloacetate transaminase	Tris-citrate	Selander *et al.* 1971	*Got-1* *Got-2*	M (70) M (70)
Phosphoglucomutase	Tris-citrate	Ayala *et al.* 1972	*Pgm*	M (30)
Esterases	Tris-citrate and Tris-borate	de Stordeur, 1976	*Est-3* *Est-4*‡	P (275) M (329)
Acid phosphatases	Tris-borate	Ayala *et al.* 1972	*Acp-1* *Acp-2*	M (114) P (53)
Fumarase	Tris-citrate	Ayala *et al.* 1972	*Fum*	M (116)
Phosphoglucoisomerase	Tris-citrate	Ayala *et al.* 1972	*Pgi*	P (238)
Tetrazolium oxidase	Tris-citrate	Ayala *et al.* 1972	*To*	M (93)

* In parentheses is indicated the size of the sample tested.
† *Ldh-1* has an unexpected property: it is revealed in the absence of PMS.
‡ Expression limited to the male sex.

Individual *Diadromus* imagos were analysed by starch electrophoresis, using two buffer systems: the discontinuous Tris-borate buffers of Poulik (1957) and the continuous Tris-citrate I buffers of Selander *et al.* (1971). Fourteen enzymic systems were assayed; they are listed in Table I.

3. RESULTS

Fourteen enzyme systems encoded by 22 loci were assayed. Among these loci, 14 were monomorphic and demonstrated a single band electrophoretic pattern in either haploid males or diploid females (Table 1).

One locus coding an esterase (*Est-4*) could be demonstrated in males only: the 329 males analysed had an active enzyme of identical electrophoretic mobility while the 79 females did not show any activity. The *Est-4* locus seems thus monomorphic but its expression is limited to males. Sex-limited esterases have been found in other Hymenoptera, but their expression was limited to females (Snyder, 1974; Tanabe, Tamaki & Nakano, 1970). This is the first report of a locus limited to the male sex.

Six loci coding an esterase (*Est-3*), an acid phosphatase (*Acp-2*), two lactic dehydrogenases (*Ldh-1* and *Ldh-2*), a sorbitol dehydrogenase (*Sdh*) and a phosphoglucoisomerase (*Pgi*) show variations in their electrophoretic patterns. *Acp-2*, *Ldh-1*, *Ldh-2* and *Sdh* loci have a phenotype with a fast band (A allozyme) or a slow band (B allozyme) in all males and some of the females, while other females have a mixed phenotype with both A and B allozymes. Similarly, all males have a single band, either fast (A) or slow (B) at the *Pgi* locus; females may be similar to males or have a three-band phenotype: the A and B bands plus a band of intermediate electrophoretic mobility.

Six different phenotypes can be recognized at the *Est-3* locus: three single-band phenotypes (A, B and C in order of decreasing electrophoretic mobility) in all males and some of the females, and three two-band phenotypes (AB, AC, and BC) in the other females.

(i) *Heredity of the polymorphic loci*

The data on the offspring of 20 virgin females are summarized in Table 2. These females produced males having each a single allozyme at the locus considered. When the mother was heterozygous about half of her sons had an allozyme of one kind, the other half an allozyme of the other kind as could be predicted if the selective values of each phenotype were equal (χ^2 calculations show no significant differences, Table 2).

We have very few data concerning the offspring of mated females: one mated female had three Pgi^A sons and one Pgi^A daughter, a second female had three Pgi^A sons and one heterozygous Pgi^{AB} daughter; an heterozygous Est^{AB} female produced three sons, one $Est\text{-}3^A$ and two $Est\text{-}3^B$, and one $Est\text{-}3^{AB}$ daughter.

(ii) *Study of a laboratory stock*

The polymorphism of four polymorphic loci (*Est-3*, *Ldh-2*, *Pgi* and *Sdh*) was studied in a laboratory stock of *Diadromus pulchellus*.

In Hymenoptera, males being hemizygous, all loci can be considered as sex linked and should behave as X-linked loci do in XY animals. Therefore, a locus will be in equilibrium when (1) the allele frequencies are equal in males and females

and (2) when the female phenotypes follow a Hardy–Weinberg distribution (i.e. when each phenotype is in the proportions p^2, $2pq$ and q^2 for a locus with two alleles).

Table 2. *Phenotype distributions at six polymorphic loci among the offspring of virgin* Diadromus pulchellus *females*

Loci	Mother genotypes	No. of mothers	Male phenotypes A	B	C	χ^2 testing disjunction
Est-3	AA	2	13	0	0	—
	BB	2	0	11	0	—
	AB	6	30	26	0	0·3 (N.S.)
	BC	3	0	12	9	0·4 (N.S.)
	AC	2	5	0	10	1·7 (N.S.)
Ldh-1	AA	1	5	0	—	—
	BB	3	0	17	—	—
	AB	6	14	18	—	0·5 (N.S.)
Ldh-2	AA	19	87	0	—	—
	BB or AB	1	0	2	—	—
Pgi	AA	17	63	0	—	—
Sdh	AA	1	5	0	—	—
	BB or AB	8	0	13	—	—
	AB	5	10	7	—	0·5 (N.S.)
Acp-2	AA	1	6	0	—	—
	BB or AB	1	0	3	—	—
	AB	4	11	6	—	1·5 (N.S.)

In the *Diadromus pulchellus* laboratory stock studied the first requisite is true for the *Est-3*, *Ldh-2* and *Pgi* loci ($P > 0{\cdot}05$), but does not apply for the *Sdh* locus ($P < 0{\cdot}05$), Table 4. The second requisite is true only for the *Pgi* locus but we observe a very important heterozygote deficiency ($P < 0{\cdot}001$) in females at the three other loci, Table 3.

4. DISCUSSION

Although enzymic polymorphism has been described in many Hymenoptera (Crozier, 1973; Tomazevski, Schaffer & Johnson, 1973; Johnson *et al.* 1969; Contel & Mestriner, 1974; Bruckner, 1974; Mestriner, 1969; Mestriner & Contel, 1972) multilocus analyses, i.e. analyses of more than three loci – remain very scarce and tend to show a low degree of genetic variability. Thus, a 24-loci analysis in three bee species (*Lasioglossum zephyrum*, *Bombus americanorum* and *Apis pura*) revealed no variation at all (Snyder, 1974) while a 7–9 loci analysis of five ant species (*Formica rufa*, *F. lugubris*, *F. truncarum*, *F. sanguinea* and *F. exacta*) showed that the polymorphism ratio is no higher than 15% (Pamilio *et al.* 1975). *Diadromus pulchellus* polymorphism appears therefore quite high for a Hymenoptera since some 30% of its loci segregate for two or more alleles. It should be noted that the studies of Snyder (1974) and Pamilio *et al.* (1975) were performed on social Hymenoptera where the environmental stress is supposed to be reduced, while

Diadromus is a non-social species in which there is strong competition especially during larval development (*Diadromus* females lay very often two or more eggs in each host, but only one develops into an imago). Therefore, we can wonder whether the low polymorphism observed in previous investigations is a consequence of the evolution of sociability rather than the consequence of haplodiploidy which characterizes Hymenoptera.

Table 3. *Observed and expected (in parentheses) phenotype distributions in* Diadromus pulchellus *laboratory stock*

Loci		Phenotypes: Females	Phenotypes: Males	χ^2 testing panmixie
Est-3	A	34 (20·61)	107	
	B	19 (7·33)	76	
	C	10 (1·53)	20	
	AB	8 (24·57)	—	
	AC	1 (11·24)	—	
	BC	0 (6·70)	—	
		72	203	101·4 ($P < 0·001$)
Ldh-2	A	16 (5·77)	43	
	B	34 (23·79)	93	
	AB	3 (23·44)	—	
		53	136	40·3 ($P < 0·001$)
Pgi	A	63 (62·98)	187	
	B	0 (0·004)	5	
	AB	1 (1·02)	—	
		64	192	0·004 (N.S.)
Sdh	A	16 (9·49)	59	
	B	17 (10·52)	112	
	AB	7 (19·98)	—	
		40	171	16·9 ($P < 0·001$)

The most interesting finding of our work is the very important heterozygote deficiency observed in *Diadromus* females. Such deficiencies have been reported in some populations of *Pogonomyrnex barbatus* and *P. badius* (Johnson *et al.* 1969; Tomazevski *et al.* 1973), but they involve only one of the polymorphic loci analysed. It is remarkable that in *Diadromus pulchellus*, the heterozygote deficiency involves the three highly polymorphic loci analysed (the *Pgi* locus, although apparently in Hardy–Weinberg equilibrium, cannot be included in this discussion because of its very slight polymorphism).

Heterozygote deficiencies are the rule in two cases: (*a*) when an enzymic locus segregates for a null allele and (*b*) when the population studied is composed of two non-interbreeding populations as is the case of sibling species (see Pasteur *et al.* 1977, for example). Both hypotheses can be dismissed on the facts that no homozygote for a null allele was found at either locus, and that *Sdh*, *Ldh-2* and *Est-3* phenotypes are independent from one another (no particular phenotype is most

frequently associated with a phenotype at an other locus, and the females heterozygous at one locus were never, but in one case, heterozygous at another locus).

Table 4. *Allele frequencies at four polymorphic loci in females and males of* Diadromus pulchellus

Loci	Allele frequencies		χ^2 testing comparison between males and females
	Females	Males	
Est-3			
A	0·535	0·527	$\chi^2 = 2{\cdot}08$ (2 D.F.), N.S.
B	0·319	0·374	
C	0·146	0·099	
Ldh-2			
A	0·330	0·316	$\chi^2 = 0{\cdot}53$ (1 D.F.), N.S.
B	0·670	0·684	
Pgi			
A	0·992	0·974	$\chi^2 = 0{\cdot}57$ (1 D.F.), N.S.
B	0·008	0·026	
Sdh			
A	0·487	0·345	$\chi^2 = 4{\cdot}64$ (1 D.F.), $P < 0{\cdot}05$
B	0·513	0·655	

Then, is the heterozygote deficiency observed in *Diadromus* due to assortative mating or to negative heterosis? None of these modes of selection have been described in Hymenoptera, but as pointed out by Crozier (1977), there are very few data on the modes of natural selection operating in this insect group.

Other explanations for the heterozygote deficiencies observed in females of *Diadromus pulchellus* involve the nature of their genetic material or its expression. (We can reject the hypothesis of a misclassification of some males as females since sexes in *Diadromus* are very different morphologically.)

Females are thought to be diploid. However, in some biparental (bisexual) species, unmated females are sometimes able to produce females (which are therefore 'haploid') among a majority of males (Moursi, 1946). In *Diadromus* there is very little chance of such 'haploid' females being produced, since Rojas-Rousse has observed only males among the offspring of 38 virgin females (1977). On the other hand, mated females can produce both sexes; eggs giving males are not fertilized at all, but are all the eggs giving females fertilized? Although it has never been demonstrated, a simple activation of the egg by a spermatozoon could be sufficient to trigger the development of a female without the occurrence of Karyogamy.

Another hypothesis to consider is the inactivation of the male genes in some females, as this seems to happen in the queen cast of natural and *in vitro* crosses between *Solenopsis xyloni* and *S. geminata* (Hung & Vinson, 1977).

Undoubtedly further analyses are needed in order to understand the population structure of *Diadromus pulchellus*, but the data we report in the present paper indicate that this species is a choice material for investigating the mechanisms of evolution in Hymenoptera.

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