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

BY NAGAT SHAUMAR,* DANIELLE ROJAS-ROUSSE
AND NICOLE PASTEUR†

Institut de Biocénétique Expérimentale des Agrosystèmes,
Université François Rabelais, Parc Grandmont, 37200 Tours (France)

(Received 30 January 1978)

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 hemi-
zygous 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 pre-
imaginal 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 × 30 × 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

* Present address: University Ain Shams, Cairo, Egypt.
† Laboratoire d’Ecologie Médicale, Faculté de Médecine and Laboratoire de Génétique
du CEREM, Université des Sciences, 34060 Montpellier, France.

https://doi.org/10.1017/S0016672300018516 Published online by Cambridge University Press
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                           | Electro-phoresis buffers | Staining procedures | Encoding loci     | Monomorphism or polymorphism* |
|-----------------------------------|--------------------------|---------------------|-------------------|-------------------------------|
| *α*-Glycerophosphate DH           | Tris-citrate             | Pasteur & de Stordeur, 1976 | a-Gpd-1, a-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             | de Stordeur, 1976    | Est-3, Est-4†      | M (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 ($\chi^2$ calculations show no significant differences, Table 2).

We have very few data concerning the offspring of mated females: one mated female had three PgiA sons and one PgiA daughter, a second female had three PgiA sons and one heterozygous PgiAB daughter; an heterozygous EstAB female produced three sons, one Est-3A and two Est-3B, and one Est-3AB 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 | $\chi^2$ testing disjunction |
|------|------------------|----------------|-----------------|----------------------------|
|      |                  |                | A      | B    | C     |       |
| *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.) |
| *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 ($P > 0.05$), but does not apply for the *Sdh* locus ($P < 0.05$), Table 4. The second requisite is true only for the *Pgi* locus but we observe a very important heterozygote deficiency ($P < 0.001$) 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 | Females | Males | \(\chi^2\) 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 Pogonomyrmex 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 | \( \chi^2 \) testing comparison between males and females |
|------|-------------------|---------------------------------------------------------|
|      | Females | Males |              | |
| *Est-3* |       |       |              | |
| A     | 0.535  | 0.527 | \( \chi^2 = 2.08 \) (2 D.F.), N.S. |
| B     | 0.319  | 0.374 |              | |
| C     | 0.146  | 0.099 |              | |
| *Ldh-2* |       |       |              | |
| A     | 0.330  | 0.316 | \( \chi^2 = 0.53 \) (1 D.F.), N.S. |
| B     | 0.670  | 0.684 |              | |
| *Pgi* |       |       |              | |
| A     | 0.992  | 0.974 | \( \chi^2 = 0.57 \) (1 D.F.), N.S. |
| B     | 0.008  | 0.026 |              | |
| *Sdh* |       |       |              | |
| A     | 0.487  | 0.345 | \( \chi^2 = 4.64 \) (1 D.F.), \( P < 0.05 \) |
| 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 (Mauri, 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.
Hymenoptera allozyme polymorphism

Part of this study was supported by a research grant in a contract between Institut de Biocén tro Ex perimentales des Agrosystèmes – E.R.A. 328 – and the University Ain Shams (Cairo, Egypt). The electrophoresis study was accomplished in the Laboratoire de Génétique du CEREM (E.R.A. 261), Université des Sciences, 34000 Montpellier (France), with the technical assistance of Mrs J. Catalan. The authors want to thank Professor V. Labeyrie who initiated this investigation and Professors L. Thaler and G. Pasteur for their useful comments.

REFERENCES

ASKEW, R. R. (1968). Considerations on speciation in Chalcidoidae (Hym.). Evolution 22, 642–645.

AYALA, J. F., POWELL, J. R., TRACEY, M. L., MOURAÔ, C. A. & PEREZ-SALAS, S. (1972). Enzymatic variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics 70, 113–139.

BRUCKNER, D. (1974). Reduction of biochemical polymorphism in honey bees (Apis mellifera). Experientia 30, 618–619.

CONTEL, E. P. B. & MESTRINER, M. A. (1974). Esterase polymorphism at two loci in the social bee. Journal of Heredity 65, 349–352.

CROZIER, R. H. (1973). Apparent differential selection at an isozyme locus between queens and workers in the ant Aphaenogaster rudis. Genetics 73, 313–318.

CROZIER, R. H. (1977). Evolutionary genetics of Hymenoptera. Annual Review of Entomology 22, 263–288.

HUNG, A. C. F. & VINSON, S. B. (1977). Interspecific hybridization and cast specificity of proteins in fire ant. Science 196, 1458–1460.

JOHNSON, F. M., SCHAEFFER, H. E., GILLASPY, J. E. & ROCKWOOD, E. S. (1969). Isozyme genotype–environment relationships in natural populations of the harvester ant Pogonomyrnex barbatus, from Texas. Biochemical Genetics 3, 429–450.

KEBB, W. E. (1967). Multiple alleles and genetic load in bees. Journal of Apicultural Research 6, 61–63.

KEBB, W. E. (1969). Some aspects of the evolution of social bees. In Evolutionary Biology (ed. Th. Dobzhansky, M. K. Hecht and W. C. Steere), vol. 3, pp. 119–175.

LABEYRIE, V. (1960). Contribution à l'étude de la dynamique des populations d'insectes. I. Influence stimulatrice de l'hôte Acrolepia assectella Z. sur la multiplication d'un hyménoptère Ichneumonidae (Diadromus sp.). Entomophaga, Memoire Hors série, no. 1, pp. 1–193.

MELTCALF, R. A., MARLIN, J. C., WHITT, G. S. (1975). Low level of genetic heterozygosity in Hymenoptera. Nature 257, 792–794.

MESTRINER, M. A. (1969). Biochemical polymorphism in bees (Apis mellifera ligustica). Nature 223, 188–189.

MESTRINER, M. A. & CONTEL, E. P. B. (1972). The Pt-3 and Est loci in the Honey bee Apis mellifera. Genetics 72, 733–738.

MOUSET, A. A. (1946). The effect of temperature on development and reproduction of Mormoniella vitripennis (Walker). Bulletin of Society Fouad 1er Entomology 30, 39–61.

PAMILIO, P., VEPSALAINEN, K. & ROSENGREN, R. (1975). Low allozymic variability in Formica ants. Hereditas 80, 293–296.

PASTEUR, G. (1974). Génétique biochimique et populations, ou: Pourquoi sommes-nous multipolymorphes. In: Polymorphisme dans le Règne animal. Mémoire de la Société Zoologique de France 37, 473–531.

PASTEUR, N., RIoux, J. A., GUILVARD, E., PECH PERIERES, J. & VERDIEZ, J. M. (1977). Existence chez Aedes (Ochlerotatus) detritus (Halliday 1833) de deux formes sympatriques et sexuellement isolées (espèces jumelles). Annales de Parasitologie humaine et comparée 52, 325–337.

PASTEUR, N. & STORDEUR, E. DE (1976). L’α-glycérophosphate-déshydrogénase du moustique Culex pipiens: génétique formelle, linkage et étude de populations. Genetica 46, 319–326.

POULIK, M. D. (1957). Starch electrophoresis in a discontinuous system of buffers. Nature 180, 1477.
POWELL, J. F. (1975). Protein variation in natural populations of animals. In Evolutionary Biology 8, 79–119.

PRALAVORIO, R., ARAMBOURG, Y. & GUENNELON, G. (1973). Essai de mise au point d’un élevage permanent d’Acrolepia assectella Zeller (Lepidoptera, Hyponomeutidae) sur milieu artificiel. Annales de Zoologie et d’Ecologie animale 5, 569–580.

ROJAS-ROUSSE, D. (1977). Influence de l’élimination des parasites en surnombre sur la survie de la descendance des femelles vierges de Diadromus pulchellus (Hyménoptère, Ichneumonide). Entomologia, Experimentalis and applicata 21, 38–50.

SELANGER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E. & GENTRY, J. B. (1971). Biochemical polymorphism in the genus Peromyscus. I. Variation of the old-field mouse (Peromyscus polionotus). Studies in Genetics, University of Texas Publications, no. 7103, pp. 49–90.

SNYDER, T. P. (1974). Lack of allozymic variability in three bee species. Evolution 28, 687–689.

STORDEUR, E. DE (1976). Esterases in the mosquito Culex pipiens pipiens: formal genetics and polymorphism of adult esterases. Biochemical Genetics 14, 481–493.

SUOMALAINEN, E. (1962). Significance of parthenogenesis in the evolution of insects. Annual Review of Entomology 7, 349–366.

TANABE, Y., TAMAKI, Y. & NAKANO, S. (1970). Variations of esterase isozymes in seven species of bees and wasps. Japanese Journal of genetics 45, 425–428.

TOMASZEWSKI, E. K., SCHAFFER, H. E. & JOHNSON, F. M. (1973). Isozyme genotype–environment associations in natural populations of the harvester ant, Pogonomyrnex badius. Genetics 75, 405–421.