ALLOZYME VARIATION BETWEEN EUROPEAN AND NEW ZEALAND POPULATIONS OF *MICROCTONUS AETHIOPOIDES*

I.I. ILINE and C.B. PHILLIPS

AgResearch, PO Box 60, Lincoln, New Zealand

Corresponding author: ilia.iline@agresearch.co.nz

ABSTRACT

The parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) was introduced to New Zealand in 1982 for control of Sitona weevil, *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), a pest of lucerne. Unfortunately, New Zealand populations of *M. aethiopoides* do not parasitise clover root weevil, *Sitona lepidus* Gyllenhal, but European populations do. Several PCR-based methods have demonstrated the existence of genetic variation between and amongst European and New Zealand populations of *M. aethiopoides*. However, enzyme electrophoresis methods have the potential to provide less expensive population markers, and these could also prove more useful than PCR-based markers for studying gene flow between European and New Zealand *M. aethiopoides* populations. This paper reports a preliminary assessment of allozyme variation between European and New Zealand *M. aethiopoides*. Nine enzymes and general proteins were assayed, but the only variation was observed at esterase (EST) with two alleles present at each of two loci.

Keywords: Euphorinae, *Sitona lepidus*, *Sitona discoideus*, intraspecific variation, enzyme electrophoresis.

INTRODUCTION

The solitary endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) has been introduced to North America for biological control of weevils in the genera *Sitona* and *Hypera* (Coleoptera: Curculionidae) (Loan 1975) and to Australia and New Zealand for control of the lucerne pest, *Sitona discoideus* Gyllenhal (Aeschlimann 1995). *Microctonus aethiopoides* was introduced to Australia from Morocco and Greece between 1977 and 1980 (Cullen & Hopkins 1982; Aeschlimann 1983a, 1995). *Microctonus aethiopoides* reared from Australian *S. discoideus* was released in New Zealand in 1982-83 (Stufkens et al. 1987) where it subsequently suppressed *S. discoideus* to below economic damage thresholds (Goldson et al. 1990; Barlow & Goldson 1993).

In 1996, a second *Sitona* species, *S. lepidus* (= *flavescens*) Gyllenhal, was discovered in New Zealand (Barratt et al. 1996) where it has since become a pest of white clover (Willoughby & Addison 1997; Willoughby & Kettlewell 1998). *Sitona lepidus* is not effectively parasitised by New Zealand's *M. aethiopoides* (Barratt et al. 1997), but parasitism of *S. lepidus* by *M. aethiopoides* has been recorded in Europe (Aeschlimann 1980; Phillips et al. 2000; Goldson et al. 2001). This difference is due to variation in the ability of *M. aethiopoides* to parasitise *S. lepidus*, rather than variation in the ability of *S. lepidus* to evade parasitism by *M. aethiopoides* (Phillips et al. 2002). Intraspecific variations in *M. aethiopoides* host range, phenology and morphology have also been observed elsewhere (Loan & Holdaway 1961; Adler & Kim 1985; Sundaralingam 1986), leading to the hypothesis that *M. aethiopoides* comprises several genetically distinct biotypes (Aeschlimann 1983b). However, such intraspecific variations could have had an environmental rather than genetic basis (Phillips et al. 1993) and robust evidence of

genetic differentiation between *M. aethiopoulos* populations has only recently been obtained (Phillips et al. 2002; Vink et al. 2003).

Introductions of European *M. aethiopoulos* are currently being considered for biological control of *S. lepidus* in New Zealand (Goldson et al. 2001). It would be useful to develop population markers for tracking the establishment and spread of European *M. aethiopoulos* if it is released in New Zealand. Also, if an arrhenotokous European population of *M. aethiopoulos* is released in New Zealand, then suitable markers would enable interbreeding with the New Zealand population to be studied. PCR-based genetic markers for separating European and New Zealand *M. aethiopoulos* have already been found (Phillips et al. 2002; Vink et al. 2003), but protein electrophoresis methods have the potential to provide very inexpensive markers that are ideal for studying inheritance patterns since allozymes are usually inherited in a Mendelian fashion. This paper describes a preliminary assessment of allozyme variation between European and New Zealand *M. aethiopoulos*.

MATERIALS AND METHODS

Thirty-six *M. aethiopoulos* lineages (separate, field-collected, female lines) from thirteen locations in Europe, Australia and New Zealand, plus twelve lineages resulting from laboratory crosses between geographic populations were studied using vertical polyacrylamide gel electrophoresis. The number, geographic origin and host species of the lineages are presented in Table 1. At least one female was studied from every lineage.

The following enzymes were assayed: dihydrolipoamide dehydrogenase (DDH, EC 1.8.1.4), esterase (EST, EC 3.1.1.-), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), glutathione reductase (GR, EC 1.6.4.2), glycerol-3-phosphate dehydrogenase (G3PDH, EC 1.1.1.8), L-idoitol dehydrogenase (IDDH, EC 1.1.1.14), isocitrate dehydrogenase (NADP) (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40) and also general proteins (GP).

Frozen whole insects were ground in 50-100 µl of one of two extraction buffers: either 0.06 M tris-HCl, pH 6.8 (EST, GP, GPI and MDH), or 0.1 M tris-HCl, pH 7.2 (EST, DDH, G3PDH, GR, IDDH and MDH). Both buffers also contained 10-15% sucrose. Samples were then centrifuged at 13 000 rpm for 5 minutes. Addition of 0.1% 2-mercaptoethanol to extraction buffers increased the resolution of the bands, but revealed no additional variation. After loading the samples onto the gels, the slabs (6-10% acrylamide gels) were run at 80 volts for 40 minutes and then run for about two hours at 250-280 volts at 4-6°C. Two electrophoretic buffer systems were used: (i) Ornstein-Davis discontinuous system (Rothe 1994) and (ii) tris-citrate pH 7.1 (Torgerson et al. 2001).

After electrophoresis the gels were stained according to Murphy et al. (1996) with the following changes: MTT was replaced with NBT; brilliant blue G was replaced with naphthol blue black for GP; 1 mg of PMS per 50 ml of staining solution was used for all dehydrogenases and GPI; β-naphthylacetate and fast garnet GBC salt were used for EST.

The most anodally migrating allozymes was designated as A, the next as B, etc.

RESULTS

This preliminary survey of nine enzyme systems plus general proteins using two electrophoretic buffer systems revealed 13 putative allozyme loci and 11 general protein bands with sufficient resolution for analysis. Eight of the enzymes (G3PDH, IDDH, IDH, DDH, GR, GPI, MDH and ME) and the 11 general proteins bands did not reveal any variation. G3PDH, IDDH, IDH, MDH and ME each had one band, while DDH, GR and GPI had two bands. Esterase was the only polymorphic enzyme and it exhibited clear variation with the presence of two loci, *Est 1* and *Est 2*, each with two alleles (Table 1). Clearer bands were obtained using β-naphthylacetate and fast garnet GBC salt rather than α-naphthylacetate and fast blue BB salt in the staining solution. Homozygotes had one band at each locus (AA or BB), and heterozygotes had two (AB), with similar overall activity as is typical for esterases and other monomeric enzymes (e.g. Murphy et al. 1996).

TABLE 1: Esterase variation (*Est 1* and *Est 2* loci) in *Microctonus aethiopoides*.

Country	Location	Host species	Lineages	<i>Est 1</i>	<i>Est 2</i>
Australia	Yanco (NSW)	<i>Sitona discoideus</i>	1	BB	AA
Finland	Mikkeli	<i>Sitona lepidus</i>	2	BB	AA
France	Caen	<i>Sitona lepidus</i>	1	BB	AA
New Zealand	Christchurch (MC)	<i>Irenimus aequalis</i> Broun ¹	2	BB	AA
New Zealand	Lincoln (MC)	<i>Listronotus bonariensis</i> (Kuschel) ¹	1	BB	AA
New Zealand	Tarras (CO)	<i>Sitona discoideus</i>	1	BB	AA
Finland/Italy	Mikkeli/Pordenone ²	<i>Sitona lepidus</i>	2	AA	AB
Finland/Italy	Mikkeli/Pordenone ²	<i>Sitona lepidus</i>	1	AB	AB
England/Romania	North Wyke/Brasov ²	<i>Sitona lepidus</i>	1	AA	BB
Ireland	Athenry	<i>Sitona lepidus</i>	1	AB	BB
Scotland/France/ Romania	Wigtown/Nancy/ Brasov ²	<i>Sitona lepidus</i>	1	AB	BB
Australia	Narranderra (NSW)	<i>Sitona discoideus</i>	1	BB	BB
England	North Wyke	<i>Sitona lepidus</i>	3	BB	BB
England/Italy	North Wyke/Pordenone ²	<i>Sitona lepidus</i>	1	BB	BB
England/Romania	North Wyke/Brasov ²	<i>Sitona lepidus</i>	4	BB	BB
France	Montpellier	<i>Sitona lepidus</i>	3	BB	BB
France	Montpellier/Nancy ²	<i>Sitona lepidus</i>	1	BB	BB
Ireland	Athenry	<i>Sitona lepidus</i>	4	BB	BB
Norway	Appelsvoll	<i>Sitona lepidus</i>	1	BB	BB
Norway	Loken	<i>Sitona lepidus</i>	2	BB	BB
Wales	Aberystwyth	<i>Sitona lepidus</i>	1	BB	BB
Wales/Norway	Aberystwyth/Loken ²	<i>Sitona lepidus</i>	1	BB	BB

¹Coleoptera: Curculionidae. ²Laboratory crosses.

DISCUSSION

Hymenoptera generally have low levels of genetic variation compared to other insects (Metcalfe et al. 1975; Pamilo et al. 1978; Graur 1985; Packer & Owen 1992; Hedrick & Parker 1997). In this study, esterase was the only polymorphic enzyme. This contrasts with results from the Argentine stem weevil parasitoid, *Microctonus hyperodae* Loan, which exhibited malate dehydrogenase variation, but no esterase variation (Iline et al. 2002). However, esterases have often been used in insect population genetics research and are generally amongst the most variable of enzymes. Even in Hymenoptera, 31.8% of assayed species were variable with an average expected heterozygosity of 8.6% (Packer & Owen 1992). Native polyacrylamide gel electrophoresis probably provides the best resolution of esterase allozymes and is widely used in insects and other taxa (Karotam et al. 1995; Scharf et al. 1997).

The results suggest that EST 2 could be used to distinguish New Zealand *M. aethiopoides* from eight of the 11 foreign populations. The exceptions were Mikkeli (Finland), Caen (France) and Yanco (Australia). The similarity with the latter population was expected since New Zealand's *M. aethiopoides* originated from Australia (Stufkens et al. 1987). In addition, the New Zealand populations differed at EST 1 from one lineage from Ireland and five lineages arising from laboratory crosses between geographic populations (Table 1). With the exception of the heterozygous lineage from Ireland, the only other lineages (n=3) that were heterozygous for EST 1 and/or EST 2 were from laboratory-crosses between populations. Further work to test whether both EST loci are fixed in the homozygous state in most natural populations of *M. aethiopoides*, as suggested by these results, would be of interest.

Esterase stains are very inexpensive and this approach may therefore offer a very practical and affordable method of tracking European *M. aethiopoides* in New Zealand.

The reliability and number of markers could be increased by studying additional enzymes, populations and specimens.

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