ALLOZYME VARIATION BETWEEN SOUTH AMERICAN GEOGRAPHIC POPULATIONS OF *MICROCTONUS* *HYPERODAE*

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ABSTRACT

Eight South American geographic populations of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) have been released in New Zealand to help suppress the pasture pest *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae). Parasitoids from each South American population were released in equal numbers throughout New Zealand. It was postulated that the population(s) best suited to the conditions encountered at each New Zealand release locality would eventually become prevalent there. Genetic markers which will enable the South American populations that have become successful in New Zealand to be identified are being developed, and this paper reports a preliminary assessment of *M. hyperodae* allozyme variation. Malate dehydrogenase (MDH) was the only variable enzyme of the six that were assayed and it exhibited variation consistent with the presence of two alleles. All parasitoids from east of the Andes (Argentina, Brazil and Uruguay) were heterozygous (MDH^a/MDH^b), while all those from the west (Chile) were homozygous (MDH^a/MDH^a). This corroborates earlier morphometric evidence of genetic differentiation between South American populations, and indicates that work with additional enzymes should yield more markers.

Keywords: Euphorinae, geographic population, intraspecific variation, enzyme electrophoresis.

INTRODUCTION

Argentine stem weevil, *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae), was discovered in New Zealand in 1927 (Marshall 1937) and is now a well known pest of pasture grasses and other Graminae in this country (Goldson et al. 1998a). The South American endoparasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae: Euphorinae) was identified as a potentially useful biological control agent of Argentine stem weevil (Loan & Lloyd 1974) and it has since established throughout New Zealand (Goldson et al. 1998b).

New Zealand's founding populations of *M. hyperodae* were collected from eight disparate locations in four South American countries (Goldson et al. 1990). These were Brazil (Porto Alegre), Argentina (Ascasubi, S. C. de Bariloche, and General Roca, Mendoza), Chile (La Serena and Concepcion) and Uruguay (Colonia).

Parasitoids from each *M. hyperodae* geographic population were released in equal numbers at each New Zealand location (Goldson et al. 1993). This ensured that parasitoids derived from each South American population had an equal chance of establishing at each New Zealand release site. It was postulated that the South American geographic populations best suited to the conditions encountered at each New Zealand release locality

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would establish there. The asexual reproduction of *M. hyperodae* (Loan & Lloyd 1974) has prevented interbreeding between the South American populations in New Zealand.

Methods of discriminating between the *M. hyperodae* geographic populations have been sought to enable the populations that have become prevalent in New Zealand to be identified. Phillips & Baird (1996) developed a morphometric method of classifying *M. hyperodae* specimens of unknown origins to one of three population groups: west of the Andes (Chile), within the Argentinian Andes (S. C. de Bariloche), or east of the Andes (Argentina, Uruguay and Brazil). Use of this method showed that parasitoids from east of the Andes had out-competed those from west of the Andes throughout New Zealand during 1992-95 (Phillips et al. 1997). Efforts to develop more precise methods of discriminating between the populations have focussed on PCR-based approaches. However, no useful population markers have been found despite the application of a comprehensive suite of methods (Winder et al. In prep.).

Allozyme electrophoresis can provide a relatively quick and inexpensive means of finding population markers, even when dealing with minute insects (Loxdale & Lushai 1998). This paper describes a preliminary survey of allozyme variation between South American geographic populations of *M. hyperodae* using frozen specimens of known origins.

METHODS

Two *M. hyperodae* specimens from each of the eight South American populations were used in each of the experiments. With the exception of specimens from Mendoza, all individuals were descended from different South American females. The two specimens from Mendoza were descended from the same South American female since only one founder was imported to New Zealand quarantine from this location.

Frozen whole insects were ground in 1-3 drops of 0.06 M tris-HCl buffer, pH 6.8, containing 10-15% sucrose, and then centrifuged at 13 000 rpm for 5 minutes. Vertical polyacrylamide electrophoresis (6-10% acrylamide gels) was conducted on the protein extracts in an Ornstein-Davis discontinuous system (Rothe 1994). The following enzymes were assayed: Esterase (EST, E.C. 3.1.1.-); general proteins (GP); glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), cytosol aminopeptidase (CAP, E.C. 3.4.11.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (malate dehydrogenase NADP) (ME, E.C. 1.1.1.40). The histochemical stains used for specific enzymes were those of Murphy et al. (1996) with the following modifications: MTT was replaced with NBT; 1 mg of PMS per 50 ml of staining solution was used for MDH, ME and GPI; and β -naphthylacetate was used for EST.

The most anodally migrating allozyme was designated as a, the next as b, etc.

RESULTS

Sufficient activity was obtained from all six enzymes to enable bands to be scored. However, MDH was the only variable enzyme and it exhibited variation consistent with the presence of two alleles. Homozygotes had one band, while heterozygotes (MDH^a/MDH^b) had three bands. These three bands had relative levels of activity that were typical of dimeric enzymes (i.e. those comprising two subunits). The middle band exhibited approximately twice as much activity as the other two bands because it resulted from the additive effects of two enzyme subunit (s.u.) combinations (i.e. MDH^a s.u.¹/ MDH^b s.u.² and MDH^a s.u.²/ MDH^b s.u.¹) (Murphy et al. 1996). All parasitoids from east of the Andes (Argentina, Brazil and Uruguay) were heterozygous (MDH^a/MDH^b), while all those from the west (Chile) were homozygous (MDH^a/MDH^a) (Fig. 1). The only population in which both heterozygotes and homozygotes (MDH^a/MDH^b and MDH^a/MDH^a) were detected was S. C. de Bariloche. The experiment with MDH was repeated three times using different specimens, and each experiment produced the same results.

FIGURE 1: Malate dehydrogenase-stained gel with *Microctonus hyperodae* specimens from Chile (homozygotes) in lanes 1, 2, 4, 6, 8 and 11. Heterozygotes from Uruguay, Brazil and two sites in Argentina are in lanes 3, 7, 9 and 10 respectively. A homozygous specimen from the mixed population in S. C. de Bariloche is in lane 5.

DISCUSSION

MDH has been among the most useful enzyme systems for studying parasitic Hymenoptera (Wool et al. 1978; Powell & Walton 1989) with about twenty percent of the species studied being polymorphic (Packer & Owen 1992). The absence of variation in the other five enzymes assayed in this study is consistent with the generally low levels of allozyme variation found in other studies of Hymenoptera (Packer & Owen 1992).

The MDH results contrast with those from a suite of PCR-based methods which have revealed almost no genetic variation in *M. hyperodae* (Winder et al. In prep.). Possible reasons for this inconsistency include: (i) variable regions of the *M. hyperodae* genome have yet to be sampled during PCR-based experiments, and (ii) differences between proteins coded by the two MDH alleles arise after translation from DNA (such variation would be difficult to detect by sequencing).

The pattern of MDH variation observed in this study is the same as that shown by a morphometric analysis (Phillips & Baird 1996), with the Andes apparently having presented a barrier to gene flow between eastern and western populations, and with a population from within the Andes (S. C. de Bariloche) being intermediate between the eastern and western populations. This pattern has also been observed in the relationship between *M. hyperodae* egg load and parasitoid size, with a strong positive correlation being exhibited by the western and S. C. de Bariloche populations, but not by the eastern populations (Phillips & Baird 2001). However, this trans-Andes genetic division has not been reflected by inter-population variations in diapause behaviour (Goldson & McNeill 1992; Goldson et al. 1993) or egg load (Phillips & Baird 2001).

The MDH results were completely reproducible and have provided further evidence of *M. hyperodae* intraspecific variation. However, many more of the 141 female lineages that were released in New Zealand (Phillips & Baird 1996) must be assayed before the MDH alleles can be confidently used as population markers. Nevertheless, the results have clearly demonstrated the potential of enzyme electrophoretic methods for providing *M. hyperodae* population markers, particularly since about one hundred enzyme systems remain to be assayed.

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