THE SOUTH AMERICAN ORIGINS OF NEW ZEALAND *MICROCTONUS HYPERODAE* PARASITOIDS AS INDICATED BY MORPHOMETRIC ANALYSIS

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SUMMARY

The Argentine stem weevil parasitoid, *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae), was introduced to New Zealand from eight South American locations. Parasitoids from seven of the South American locations were liberated in equal numbers at each release site in New Zealand during 1991 to give each population an equal opportunity to establish. Morphometric analysis of *M. hyperodae* adults allows their South American derivation to be broadly defined. This contribution reports the results of a morphometric analysis of *M. hyperodae* retrieved from four release sites in New Zealand approximately 2 years after the insect's release. The ecological implications of these results are briefly discussed.

Keywords: *Microctonus hyperodae*, morphometric analysis, biological control

INTRODUCTION

Microctonus hyperodae Loan (Hymenoptera: Braconidae, Euphorinae) was introduced to New Zealand in 1991 as a biological control agent of Argentine stem weevil (*Listronotus bonariensis* Kuschel (Coleoptera: Curculionidae)), a major pasture pest. This koinobiontic, thelytokous parasitoid was collected from eight South American locations (Fig. 1; Goldson *et al.* 1990). Progeny of seven geographic populations were subsequently released in equal numbers at thirteen New Zealand release sites (Goldson *et al.* 1994), while progeny of a single female collected from Mendoza were also released at the same sites in smaller numbers. This was aimed at maximising the genetic diversity of *M. hyperodae* to facilitate its establishment and success in New Zealand. It was hypothesised that the populations most suited to the conditions prevailing at each release site would out compete those that were less 'pre-adapted' and eventually become dominant there (eg. Goldson *et al.* 1993).

Methods of separating and identifying descendants of each South American population are being developed so that their establishment and spread in New Zealand can be monitored. Eventually, knowledge of the parasitoids' South American origins combined with a growing understanding of the South American populations' unique behavioural characteristics (Goldson and McNeill 1992; Goldson *et al.* 1993) should allow the definition of some of the factors which predisposed them to success or failure in various parts of New Zealand. This should contribute to biological control theory and practice. Also, comparison of the results of different discriminatory methods may usefully demonstrate their respective strengths and weaknesses.

A first attempt to develop methods of discriminating between the geographic populations involved a morphometric analysis of *M. hyperodae* adults of known South American origins (Phillips, unpublished data). This analysis revealed that significant morphometric differences occurred between the South American populations and that the differences were greatest between the populations derived from opposite sides of the Andes. Conversely, the *M. hyperodae* populations from near the east coast of South America were the most difficult to separate on the basis of their morphology. The

Figure 1: Map of South America showing locations where *M. hyperodae* was collected for release in New Zealand.

population from Bariloche, which is located in the Andes, most closely resembled the Concepcion and La Serena populations. These results meant that errors were more likely to occur when classifying a specimen of unknown origins into a population in the group comprising Ascasubi, Colonia, Porto Alegre, and Rio Negro (Fig. 1). On the other hand, a specimen classified as belonging to a population in the group comprising Ascasubi, Colonia, General Roca, Mendoza and Porto Alegre (henceforth called the "east of the Andes" group) was very unlikely to belong to one of the populations in the group comprising La Serena and Concepcion (henceforth called the "west of the Andes" group), and vice versa (Figure 1). Therefore, the morphometric method (Phillips in preparation) was found to have the potential to provide a broad indication of the South American origins of *M. hyperodae* collected in New Zealand.

The results of a morphometric analysis of *M. hyperodae* collected from four New Zealand release sites in 1993 are presented and discussed in this contribution. The limitations of the method, combined with the small number of *M. hyperodae* available from the New Zealand sites for assessment, indicated that the most robust analysis would involve a two way classification of New Zealand specimens into the "east of the Andes" and "west of the Andes" groups. For the purposes of this contribution, progeny of the single Mendozan female were assumed to have been released in the same numbers as progeny of parasitoids collected from the other seven locations. The null hypothesis (H_0) was that, in the absence of any differential selection of the populations, all of the South American geographic populations would be equally represented in random samples of *M. hyperodae* in New Zealand. Under H_0 therefore, the "west of the Andes" South American populations were expected to comprise 3/8 and the "east of the Andes" populations 5/8 of *M. hyperodae* in New Zealand.

METHODS

Adult Argentine stem weevils were collected from sites where *M. hyperodae* was released during 1991 (Goldson *et al.* 1993) at Lincoln (Canterbury), Reporoa, Ruakura (Waikato) and Wellsford (Auckland). Collections were made during winter because *M. hyperodae* are more or less synchronised as larvae within adult weevils at this time (Barlow and McNeill, pers. comm.). The collection details are presented in Table 1; the methods noted in Table 1 have been described more fully by Proffitt *et al.* (1993).

The weevils were stored in cages at approximately 20°C, 12:12 L:D and any parasitoids that emerged were reared to the adult stage as described by Goldson and Proffitt (1990). *M. hyperodae* adults to be used for morphometric analysis were stored in 70% alcohol or put in a -70°C freezer prior to being placed in alcohol solution.

TABLE 1: *M. hyperodae* release date, collection site, collection date, collection method, and number of parasitoids assessed.

NZ release site	Date *M. hyperodae* first released	Date parasitised ASW collected	Collection method	No. of parasitoids assessed
Lincoln	11 April 1991	25 May 1993	heated pad	6
Lincoln	11 April 1991	10 August 1993	vacuum	16
Reporoa	12 April 1991	31 August 1993	vacuum	20
Ruakura	12 April 1991	25 August 1993	vacuum	7
Wellsford	12 May 1991	25 May 1993	vacuum	9
Wellsford	12 May 1991	25 August 1993	vacuum	13

Adult parasitoids were assessed by measuring 14 morphological variables (Phillips in preparation). The characters used were: total number of segments in both antennae; lengths of antennal segments 1 and 2 of one antenna; length of one antenna; length of basitarsus 3; width of metasomal tergum 1 in dorsal view at its widest point (posterior margin), narrowest point, and along the anterior margin; length of the radial cell; length of the costal vein of the radial cell; length of the stigma; width of the stigma; width of the forewing; length of the forewing.

The morphometric data of *M. hyperodae* specimens of known South American origins (Phillips unpublished data) were used to develop morphometric criteria by which to classify specimens of unknown origins. The classification criteria were computed using the SAS procedure DISCRIM to create mathematical functions into which the morphometric data from each specimen could be substituted. These quadratic discriminatory functions were calculated so that the morphometric data of two specimens from the same population tended to give similar results (discriminatory scores), while the data of specimens from different South American populations tended to give different discriminatory scores. The morphometric data of the New Zealand specimens were then substituted into the same discriminatory functions. The discriminatory score of each New Zealand specimen was compared to the mean score of each South American population calculated from the data of specimens of known origins. By taking into account the respective distributions of the South American populations' scores, as well as the "distance" between the New Zealand specimen's score and the eight South American mean scores, the New Zealand specimen was classified as belonging to one of the South American populations. The number of discriminatory functions that could be developed was equal to the number of groups to be separated minus one. Therefore, seven functions were used in this analysis, although the first few generally accounted for most of the inter-population variability.

Each New Zealand specimen was initially classified to its single most probable population of origin and these were then combined into the "east of the Andes" and "west of the Andes" categories. The proportions of New Zealand specimens classified to each of the South American groups were compared to those expected under H_0 using a log linear model and Poisson errors (McCullagh and Nelder 1983) in a Genstat (version 5.3) program. The significance of any departure from the proportions predicted under H_0 was assessed using a likelihood ratio statistic (l); -2log(l) was compared with its asymptotic distribution (Chi-squared with n degrees of freedom where n is the number of parameters in the alternative hypothesis) to obtain the probability under H_0.

RESULTS

A total of 71 *M. hyperodae* females collected in New Zealand were assessed (Table 1), and the number classified by discriminatory analysis as belonging to each South American population are given in Table 2. Classifications of New Zealand specimens into individual South American populations are tentative, while those into the "east of the Andes" and "west of the Andes" groups are likely to be more robust for the reasons described above.

TABLE 2: Number of *M. hyperodae* assigned to each South American geographic population from each New Zealand collection site. The number expected under H_0 are in brackets (ie. the number of specimens collected from each New Zealand site divided by eight).

	Lincoln (2.8)	Reporoa (2.5)	Ruakura (0.9)	Wellsford (2.8)	Total (8.9)
Ascasubi	5	3	0	4	12
Porto Alegre	3	2	0	2	7
Mendoza	2	2	1	1	6
Rio Negro	6	6	3	10	25
Colonia	5	6	2	1	14
Total "E. of Andes"	21	19	6	18	64 (44)
Bariloche	1	0	1	1	3
La Serena	0	0	0	2	2
Concepcion	0	1	0	1	2
Total "W. of Andes"	1	1	1	4	7 (27)
Overall total	22	20	7	22	71

There was no significant difference between the New Zealand collection sites in the proportions of *M. hyperodae* specimens assigned to each South American geographic population. However, over all of the New Zealand collection sites, a significantly greater proportion than expected was classified as belonging to the South American "east of the Andes" group of populations ($P<0.001$).

There was no significant difference between the proportions of specimens assigned to each of the three "west of the Andes" South American populations. However, among the five "east of the Andes" populations, there were significantly more specimens classified as belonging to the *M. hyperodae* population derived from Rio Negro ($P<0.001$). There was no significant difference in the proportions of specimens classified as belonging to the populations from Ascasubi, Porto Alegre, Mendoza and Colonia.

The discriminatory scores from the first two of the seven discriminatory functions used to classify the New Zealand specimens are presented in Figure 2. Each New Zealand specimen assessed in this study is plotted on the graph, along with the mean scores for each South American population taken from the "calibration" database. This graph illustrates how the majority of New Zealand specimens assessed in this study were morphologically most similar to *M. hyperodae* derived from Ascasubi, Colonia, Porto Alegre and Rio Negro. The differences between the discriminatory scores of the

New Zealand specimens and the mean scores of the populations to which they were classified, were similar to the differences between the discriminatory scores of the specimens of known South American origins and the means of the populations to which they belonged.

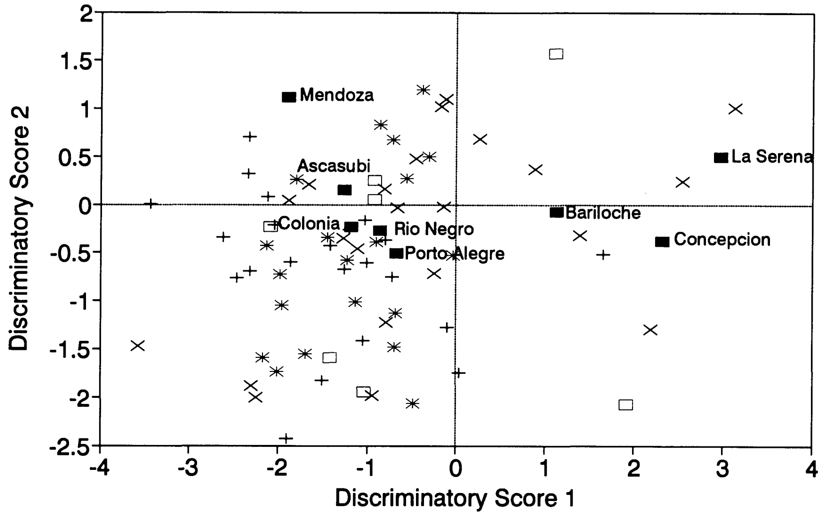

Figure 2: Graph of the scores of the first discriminatory function versus those of the second function. Individual specimens from New Zealand are marked (+ Lincoln; ✳ Reporoa; □ Ruakura; × Wellsford) as are the mean scores for each South American geographic population (■).

DISCUSSION

The results of this study are based on two major assumptions. The first is that a random sample was taken of the South American geographic populations of *M. hyperodae* present at each New Zealand site. This was supported in part by the observation that specimens from each New Zealand site were classified to the various South American geographic populations in similar proportions (Table 2). This suggests that the samples were not biased by variables in the collection process such as dates and methods (Table 1). The assumption was further strengthened by collecting immature *M. hyperodae* during winter. The phenological synchrony of the parasitoids which occurred at this time (Barlow and McNeill pers. comm.) probably eliminated any bias that could have resulted from sampling only one life stage of the insect.

The second assumption is that *M. hyperodae* exposed to New Zealand field conditions have not changed significantly (in morphometric terms) from the laboratory-reared specimens used to create the "calibration" database. This assumption was supported by the fact that the discriminatory scores of the New Zealand specimens were similar to, and had the same distribution as, those derived from the laboratory-reared insects of known South American origins. If New Zealand field conditions had significantly influenced *M. hyperodae* adult morphology, then spurious discriminatory scores would have been likely to occur. The opportunity for environmental factors to cause morphometric variations (eg. Phillips *et al.* 1993 and references therein) in *M. hyperodae* adults in this study was probably reduced by rearing the insects from the larval stage under standard laboratory conditions.

The preliminary data presented in this study suggest that parasitoids descended from the "east of the Andes" South American populations comprised a greater proportion than expected of the *M. hyperodae* occurring at four New Zealand release sites during the winter of 1993. Thus, biological differences between some of the *M. hyperodae* geographic populations imported to New Zealand (Goldson and McNeill 1992; Goldson *et al.* 1993) seem to have become evident in the field only 2 years after the parasitoid's release (Table 1). Although insects classified specifically as being derived from Rio Negro were also significantly more numerous in the New Zealand samples, this result remains much more tentative. Figure 2 illustrates how close together the mean scores are of the first two discriminatory functions for the populations from Ascasubi, Colonia, Rio Negro and Porto Alegre. Although the remaining five discriminatory functions tend to improve the separation of these populations (Phillips unpublished data), insects descended from these populations are still more likely to be misclassified within this grouping when categorised on a morphometric basis.

Earlier research on *M. hyperodae* suggests two possible reasons for the apparent early success of one or more South American "east of the Andes" populations. Goldson and McNeill (1992) and Goldson *et al.* (1993) reported that the parasitoid population from Porto Alegre in Brazil (Fig. 1) tended to display a much weaker diapause response under short day lengths than those from the other South American localities. Thus, it is possible that the reduced diapause response of this population has been advantageous under New Zealand conditions, at least during the species' establishment phase in this country. A second possibility has been pointed to by Williams *et al.* (1994). They showed that New Zealand's Argentine stem weevil founder population was most likely to have come from the east coast of South America, probably in the vicinity of Uruguay or Buenos Aires. Therefore, it is possible that parasitoids from one or more of the "east of the Andes" populations have some selective advantage because they coevolved until recently with the host weevils which occur in New Zealand. To date however, any such advantage of *M. hyperodae* from one population over those from another has proved difficult to define in laboratory-based studies (McNeill and Phillips unpublished data).

Research into the development of biochemical methods of differentiating between the South American geographic populations is currently underway (Williams and Phillips unpublished data). When completed, these methods, in combination with the morphometric approach used in this study, should provide more precise information about the South American origins of the *M. hyperodae* populations which become prevalent in New Zealand. This may facilitate our understanding of the importance of factors such as variation in diapause behaviour and host/parasitoid coevolution to biological control outcomes.

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